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The Interaction of Chloroquin, Dilantin, and Phenylbutazone on DDT Storage in the Rat

38 p.

Food and Drug Administration
Chamblee, Georgia 30341

M. F. Cranmer, Ph.D. and A. Peoples, D.H.E.W., P.H.S., F.D.A.,
Division of Pesticides, Perrine Primate Research Branch, Perrine, Florida

The purpose of the experiment was to compare the effects of dilantin, chloroquine and phenylbutazone on the storage of p,p'-DDT in the rat. Eleven groups of six animals were administered drugs in their feed for ten weeks. At the time of sacrifice, liver microsomal enzymes, including the 0-demethylation of p-nitroanisole, oxidative detoxification of EPN, azo-reductase, expoxidase, glucuronal transferase and cytochrome P-450, were determined. The fat of all animals was examined for p,p'-DDT and p,p'-DDE in the fat of the animals; however, the degree of reduction was not identical for the three drugs, and the reduction due to combination treatments was not that which would have been predicted had the treatment effects been additive. The three drugs exhibited different inductive effects on the several hepatic microsomal enzymes measured.

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Comparison of Chronic and Acute Low-Level Exposure Effects of Carbaryl on the EEG of Squirrel Monkeys

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Food and Drug Administration
Chamblee, Georgia [illegible]

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The purpose of the present experiment was to compare the effects on the anesthetized electroencephalogram (EEG) of low-level, chronic carbaryl feeding with those resulting from a single i.v. injection in Squirrel monkeys.

Six chronic monkeys have received 0.007 mg/kg carbaryl per day in a miniature marshmallow for 26 months. Their EEG's were compared to those of 6 contemporary control animals receiving a daily marshmallow without pesticide. Acutely dosed monkeys were intravenously given 6 mg/kg carbaryl in 0.15 ml 95% ethanol. Their pre- and post-injection EEGs were compared.

All EEG recordings were made with subcutaneous needle electrodes positioned at mid-frontal (MF) and right (RO) and left (LO) occipito-parietal locations. Animals were anesthetized with Surital. In addition to an oscillographic record, an interval histogram was constructed from the MFxRO signal using a Lab-8 computer.

Both acute and chronic monkeys exhibited a decreased and an increased slow-wave amplitude, and an increased variability of pattern compared to non-treated records. Common to both treatment groups also was a slight decrease in bilateral symmetry of the EEG.

The post-injection interval histogram of acutely dosed animals showed a distinct shift toward shorter intervals compared to their pre-injection histogram. This shift was not seen in the chronic-dosed monkeys. Although the slow-wave amplitude was decreased in both treatment groups, the chronic-dosed monkeys showed an increase in the abundance of intervals longer than 1.5 seconds while the acutely dosed animals did not.

It is concluded that low-level chronic exposure of Squirrel monkeys to carbaryl results in detectable changes in the anesthetized EEG pattern and that several but not all of these changes are seen in acutely dosed monkeys.

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Environmental Protection Agency
Chamblee, Georgia 30341

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142. *Effects of o,p′-DDT on the Adrenal Cortex of the Beagle.* M. F. COPELAND and M. F. CRANMER, Environmental Protection Agency, Perrine, Florida.

The purpose of this study was to investigate the influence of the continued po administration of *o,p′*-DDT on the adrenal cortex of the beagle. Four of eight males received *o,p′*-DDT (50 mg/kg/day) dissolved in corn oil and administered in gelatin capsules; four received corn oil only. Plasma sodium, potassium and 17-hydroxycorticosteroids (17-OHCS), as well as hematocrit and circulating eosinophils, were determined before and after iv administration of 40 units of ACTH on each dog at intervals during the 32 days of treatment. After 32 days the animals were necropsied, and histological studies were performed on the adrenals of all dogs and adrenal 17-OHCS levels determined. GLC determinations were made of *o,p′*-DDT and its metabolites in adrenal, liver, testis, and adipose tissue. The treated dogs maintained body weights comparable to those of the controls, though treated dogs showed a decreased eosinopenic response to ACTH after day 16, when compared to controls. Two-way classification analysis of variance showed no significant difference at $p < 0.05$ in the 17-OHCS response to ACTH nor within group variation between treated and control dogs. There was no significant difference at $p < 0.05$ in plasma sodium and potassium levels, nor in the hematocrit in the treated as compared to controls before or 4 hr after ACTH on any of the test days, nor in the adrenal 17-OHCS levels at the conclusion of the experiment. The adrenals of the treated dogs were significantly larger ($p < 0.01$) than those of the controls. Histological studies showed cells in the fasciculata of the treated dogs to be larger, more vacuolated, and devoid of acidophilic cytoplasm with hyperchromatic nuclei, which frequently occupied a peripheral position in the cell. Of the tissues analyzed only fat contained a higher level of *o,p′*-DDT than the adrenals in treated dogs. Results from this study suggest that *o,p′*-DDT does not block the synthesis of corticosteroids by the adrenal cortex in the beagle dog as does *o,p′*-DDD.

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2. *The Electroencephalogram and Visual Evoked Potential of the Squirrel Monkey Fed Hexachlorophene.* J. A. SANTOLUCITO, Environmental Protection Agency, Perrine, Florida.

Young adult, female Squirrel monkeys were administered po hexachlorophene in oil daily for 28 days. Two monkeys were placed on each of the following treatments: oil only, and 1, 5 or 15 mg/kg body wt. Following the treatment period one animal of each group was sacrificed. The four remaining animals were held an additional 28 days without further treatment before sacrificing. Before, and at intervals throughout the experiment, EEG recordings were obtained; just prior to sacrifices an averaged visual evoked potential to light flash was taken. Electrode placements used were right and left occipito-parietal (RO and LO) and midfrontal (MF). On-line computer processing of the brain biopotentials was performed using a Digital Equipment Corp. LAB-8. Microscopic examination of brain and spinal cord was performed by Dr. Kimbrough, Chamblee Toxicology Laboratory, Perrine Primate Laboratory. At the end of 28 days of treatment the primary component of the visual potential was attenuated at all three dose levels. This effect was still seen in those animals removed from treatment for 28 days. The EEG underwent a decrease in mean frequency throughout the treatment and posttreatment periods in all animals, suggesting a possible influence of repeated anesthesia. However, the decrease in hexachlorophene monkeys was approximately twice that of controls. No differences were seen in degree of bilateral correspondence of spontaneous electrical activity attributable to hexachlorophene. Histology of the CNS did not reveal lesions seen previously in rats given high doses of hexachlorophene. In view of the limited data, we regard these findings as tentative. Nevertheless, they appear suggestive enough to warrant further studies on the feasibility of using EEG as an early indicator of the effects of hexachlorophene.

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Food and Drug Administration
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TOXICOLOGY AND APPLIED PHARMACOLOGY, 17(1):315, JULY 1970

Effect of Diphenylhydantoin on Storage of DDT in the Rat. M. F. CRANMER, Food and Drug Administration, Perrine Primate Research Branch, P.O. Box 490, Perrine, Florida.

Dilantin is a widely used anticonvulsant drug which has been shown to stimulate liver microsomal enzymes and reduce DDT and DDE residues in man. Our investigation was undertaken to study the effect of Dilantin on the storage of DDT and DDE in the rat.

One hundred and forty-four female rats were divided into three groups and received 5, 10, or 15 ppm p,p′-DDT in their diet for 3 weeks. One-half of each group received 250 ppm Dilantin plus their respective doses of p,p′-DDT for the second 3 weeks of treatment. Four rats of each treatment group were sacrificed at the end of weeks 3, 4, 5, and 6. At the beginning of week 7 p,p′-DDT was removed from the diet of all rats, and four animals of each group were sacrificed at the end of week 7, 8, and 9. Levels of p,p′-DDT and DDE in fat were determined for each sacrificed animal. Liver microsomal enzyme activity was measured for those animals sacrificed at the end of the sixth and ninth weeks of treatment.

A second group of 24 rats was fed 10 ppm p,p′-DDT for 14 months. Half the group was sacrificed, and the remaining 12 animals were fed 10 ppm p,p′-DDT and 250 ppm Dilantin for 6 weeks prior to sacrifice. Levels of p,p′-DDT and p,p′-DDE in fat and liver microsomal enzyme activity were measured for all 24 animals.

Dilantin was found to reduce the levels of p,p′-DDT and p,p′-DDE in the fat and to increase liver microsomal enzyme activity in all animals except those receiving 10 ppm p,p′-DDT for 14 months.

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Chamblee, Georgia 30341

12. *Observations on the Alteration by Carbaryl of Smooth Muscle Preparation Responses to Norepinephrine.*

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Previous work from this laboratory (Hassan) suggested that orally administered carbaryl caused an increased synthesis and release of norepinephrine in rats. The present experiment was an attempt to determine whether carbaryl interacts directly with adrenergic mechanisms. Motility response patterns to norepinephrine (NE) in conjunction with alpha- and beta-receptor blocking agents were recorded from isolated preparations of uteri from ovariectomized rats and intestinal segments from rats and rabbits. Nonestrogenized uterus motility was inhibited by 4×10^{-6} M NE or 1×10^{-4} M carbaryl; 7×10^{-5} M phentolamine (PHEN), an alpha-blocking agent, did not abolish the inhibitory action of either compound. Estrogenized uterus motility was stimulated by NE alone and inhibited by PHEN + NE. Carbaryl did not modify this response. 4×10^{-4} M carbaryl antagonized the inhibitory effect of NE on intestinal motility, as did 7×10^{-5} M PHEN. The beta-blocking agent propranolol (PRO), 4×10^{-6} M, did not antagonize the inhibitory effect of NE. It is concluded that carbaryl can interact with adrenergic receptors behaving as a beta-sympathomimetic in uterine preparations and as an alpha-blocking agent in intestinal segments.

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TOXICOLOGY AND APPLIED PHARMACOLOGY, 14(3):621, MAY 1969

Lack of Correspondence at High Acute Doses of DDT between the Levels Found in Blood and Brain. J. SANTOLUCITO, C. CUETO, JR., E. WHITCOMB, and J. OWENS, Pharmacology Section, Pesticides Research Laboratory, Pesticides Program, Food and Drug Administration, Perrine, Florida.

It is generally acknowledged that the concentration of DDT in the fat does not reflect the amount in brain tissue and therefore does not correlate with acute toxic symptoms. While some investigators have reported that plasma levels tend to correlate with brain levels, others report that no correlation exists. The present study is an attempt to elucidate this question.

In the first experiment 18 mature, male, Sprague-Dawley rats were orally dosed with 200, 400, or 800 mg/kg *p,p'*-DDT. Three animals were treated as a set each day, each dose being represented in every set. All animals were sacrificed at the time of severe tremor and/or convulsion. In the second experiment, another group of 16 rats was orally dosed with 800 mg/kg *p,p'*-DDT, and two animals were sacrificed every 30 min. At the time of sacrifice, blood, cerebral hemispheres, cerebellum, and brain stem were recovered for analysis of DDT by gas chromatography. The results of the first experiment revealed no differences in blood and brain content of DDT due to treatment. On the other hand, replication mean squares were consistently larger than treatment mean squares. The F ratio for replications was highly significant for the three brain regions. Cerebral hemispheres contained less DDT at the time of convulsion than did the other two regions at all dose levels studied. The time to convulsion varied inversely with dose level. In the second experiment, the regression of DDT concentration against time showed similar rates of accumulation for all three brain regions. Correlation coefficients were 0.75, 0.80, and 0.74 for cerebellum, cerebral hemispheres, and brain stem, respectively. The blood concentration, on the other hand, reached a plateau between 30 min and 1 hour and remained constant for the 4 hours of the study.

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Technology Branch
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Paralytic and Related Effects of Certain Organic Phosphorus Compounds

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The use of organic phosphorus compounds as pesticides has become quite important. The amount of these compounds used for insecticidal purposes is now exceeded only by the tonnage of chlorinated hydrocarbon materials.

It has been generally recognized that these organic phosphorus compounds possess a high degree of toxicity. Early reports concerning the deleterious effects of insecticides belonging to this class dealt with signs and symptoms resulting, at least in large part, from cholinesterase depletion following single or repeated exposures. The consensus was that if an exposed organism survived the acute effects of the poison it would recover with no residual effects.[1]

However, as early as 1930 Smith and his co-workers * established that TOCP (triorthocresyl phosphate), which had been used to fortify certain lots of extract of Jamaica ginger, was the etiological agent in a large number of cases of paralysis occurring in the United States. Smith and Lillie[4] showed that this so-called "ginger paralysis" or "jake leg" was accompanied by a demyelination of the nerve sheath. The anticholinesterase activity of TOCP was not established until much later.[5] Polyneuritis resulting from the poisoning of industrial workers with TOCP was reported in 1944.[6]

In 1946 Koelle and Gilman[7] demonstrated muscle weakness of the hindlegs, eventually leading to almost complete paralysis, following repeated sublethal doses of DFP (diisopropyl fluorophosphate) in dogs. In 1949 these same authors[8] postulated the identity of this syndrome with "ginger paralysis." They further suggested that the syndrome of "ginger paralysis" might be a reflection of the effect of prolonged deficiency of cholinesterase at the neuromuscular junction.

In 1951 Petry[9] described paralysis in a man who had been using parathion in greenhouses in Germany and who had previously experienced mild symptoms of poisoning on several occasions. Although the mode of onset and subsequent course of this case of paralysis closely resembled "ginger paralysis," it occurred almost three months after the last known exposure to parathion. The unusually long interval of time between exposure and the onset of paralysis casts doubt on the validity of indicting parathion as the etiological agent in this unique case.

Later in 1951 a group of three chemical plant workers who were engaged in the manufacture of Isopestox (bis-isopropylaminofluorophosphine oxide) † developed acute cholinergic symptoms attributed to intoxication with this compound.[10] Their acute symptoms were ameliorated by atropine, but 10 days later two of the three developed weakness in the legs. In one the weakness progressed to almost complete

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From the Communicable Disease Center, Public Health Service, U. S. Department of Health, Education, and Welfare.

* References 2 and 3.

† Mipafox, common name recommended by the British Standards Institution.

paralysis of the legs, with partial involvement of the hands and arms. Ten months later both had residual effects. The more severely affected was still unable to walk two years later.[11]

With Isopestox, as with TOCP, it was found that the chicken was the experimental animal most susceptible to delayed paralytic effect. Table 1 gives a summary of the results of previous studies on the paralytic effect of organic phosphorus compounds in chickens.

Barnes and Denz [12] demonstrated demyelination in the peripheral nerves and particularly in the spinal cord of chickens paralyzed by TOCP, DFP, and Isopestox. The similarity of the histological lesions in hens poisoned by each of the three compounds was pointed out.

Table 1.—*Summary of Results of Previous Studies on the Paralytic Effect in Chickens of Various Organic Phosphorous Compounds*

Common Name	Chemical Name	Paralysis	Authority
DFP	diisopropyl fluorophosphate	Yes	12, 16, 17
Ethyl sarin	O-isopropyl ethyl fluorophosphonate	No	13
Isopestox	bis-isopropylaminofluorophosphine oxide (bis-isopropylamido fluorophosphate)	Yes	12, 16, 17
Isopropyl OMPA	tetraisopropyl pyrophosphoramide	No	12, 17, 18
Isopropyl paraoxon	O,O-diisopropyl O-*p*-nitrophenyl phosphate	No	17
Isopropyl parathion	O,O-diisopropyl O-*p*-nitrophenyl thiophosphate	No	12
Methyl parathion	O,O-dimethyl O-*p*-nitrophenyl thiophosphate	No	12
Paraoxon	O,O-diethyl O-*p*-nitrophenyl phosphate	No	12
Parathion	O,O-diethyl O-*p*-nitrophenyl thiophosphate	No	12
Sarin	O-isopropyl methyl fluorophosphonate	No	13
Soman	O-(2,2-dimethylbutyl-3) methyl fluorophosphonate	No	13
Tabun	dimethylamido O-ethyl cyanophosphate	No	13
TIPP	tetraisopropyl pyrophosphate	No	12, 17
TOCP*	triorthocresyl phosphate	Yes	12, 14, 16, 17

* For analogues of TOCP, see Ref. 13.

Studies attempting to correlate the ability of different compounds to cause delayed paralysis, with chemical structure, various physical properties, and pharmacological effects, including particularly pseudocholinesterase inhibition, have given no leads on the types or classes of phosphates which could be expected to possess this toxic action.‡ Of a series of TOCP derivatives, Hine and associates [14] found that only those triaromatic substituted phosphates containing at least one ortho-positioned methyl group caused paralysis. Although no correlation between paralytic effect and cholinesterase inhibition has been found, all compounds known to produce the paralysis do inhibit plasma cholinesterase. A metabolic antagonism to vitamin E has been suggested as the etiological mechanism for the production of paralysis by TOCP.[15]

In view of the serious and essentially irreversible nature of this paralytic syndrome and the widespread usage of various organic phosphorus compounds as insecticides, it was thought desirable to screen the various insecticides of this group now being marketed for ability to cause "ginger paralysis." It is perhaps even more important that all new compounds with this grouping should be screened for this effect before being admitted for general usage. The paralytic effect in the chicken appears to be the best available index of a possible paralytic effect in man.

METHOD

The following method was used to screen compounds. Five groups of two chickens each were dosed subcutaneously with geometrically increasing doses of the phosphate to be tested. Adult hens of the Rhode Island Red strain weighing between 2.5 and 4.0 kg. were used. The insecticides were given in peanut-oil solution at the rate of 1 ml/kg. of body weight. Dosages were adjusted by varying the concentration of toxicant in solution. Each chicken was predosed orally with atropine sulfate at the rate of 15 mg/kg. in order to minimize acute cholinergic effects. The mortality of these birds after an observation period of one week was noted,

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‡ References 13; Edson, E. F.: Personal communication to the authors.

and an approximate L. D.$_{50}$ derived. A larger group of atropinized chickens was then dosed with the test material at a level slightly below the estimated L. D.$_{50}$. All dosed chickens were held for at least 30 days and observed daily, with particular attention being given to the legs. The toxic symptoms were best observed by allowing each chicken to stand or walk on an irregular surface.

RESULTS

The results for the compounds tested in this manner are shown in Table 2. Compounds tested by other workers were not included, except TOCP and Isopestox. Groups of chickens dosed with these two compounds were included for comparison, and these animals, as expected, regularly developed paralysis of the legs about 10 to 14 days after dosing.

TABLE 2.—*Toxic Effects in Atropinized Chickens of Selected Organic Phosphorus Compounds When Given Subcutaneously in Oil Solution*

				Animals Showing Stated Effect					
Compound	Dosage Range, Mg./Kg.	Lowest Dosage Producing Cholinergic Signs, Mg./Kg.	Animals Dosed, No.	Acute Cholinergic Signs, No.	Immediate Muscle Weakness, No.	Delayed Paralytic Effect, No.	Death	Average Survival Time,* Days	Approximate L. D.$_{50}$, Mg./Kg.
Chlorthion	100-1,600	400	20	15	0	0	4	9	1,200
DDVP	10-160	10	18	18	0	0	8	1	20
Demeton	5-80	10	20	6	0	0	5	1	20
Diazinon	5-80	10	20	18	0	0	11	1	15
EPN	5-1,280	40	28	21	21	0	18	18	60
Isopestox†	60	>60	3	0	0	3	..‡	..	.....
Malathion	50-1,600	100	24	21	8	0	4	2	1,400
OMPA	10-320	20	24	21	0	0	5	1	200
TOCP	50-400	200	10	0	0	5	4	21	200

* Based on those animals which died.
† All dosages of Isopestox were given orally.
‡ Two hens killed for pathological study when in a moribund state.

Some acute cholinergic effects were seen at higher dosage levels with all compounds tested. No delayed effects were seen following dosage with

Chlorthion	O,O-dimethyl *p*-nitro-*m*-chlorophenyl thiophosphate
DDVP	O,O-dimethyl dichlorovinyl phosphate
Demeton	O, O-diethyl 2-ethylmercaptoethyl thiophospate
Diazinon	O, O-diethyl 2-isopropyl-4-methylpyrimidyl-6 thiophosphate
OMPA	octamethyl pyrophosphoramide

A sydrome different from "ginger paralysis" appeared in the hens dosed with

Malathion	O,O-dimethyl S-(1,2-dicarbethoxyethyl) dithiophosphate
EPN	O-ethyl phenyl *p*-nitrophenyl thiophosphonate

These chickens developed leg weakness immediately after dosing in addition to the more usual cholinergic symptoms. The occurrence of general muscle weakness and incoordination as a nonspecific sign in acutely ill, poisoned animals is not unusual. However, the muscle weakness occurring in chickens dosed with malathion and EPN was localized and persisted after the other acute symptoms had vanished. While present, this weakness was clinically indistinguishable from "ginger paralysis." For the purposes of Table 2, only immediate muscle weakness lasting longer than 48 hours has been considered under that heading.

Muscle weakness occurred in 6 out of a group of 10 chickens dosed with malathion at 1000 mg/kg. Two other hens in this group died from acute malathion poisoning in less than 48 hours, and the remaining two developed no signs of illness. The lowest malathion dosage giving muscle weakness was 100 mg/kg. The minimum dosage tested was 50 mg/kg. At this level neither of the chickens was affected. The localized muscle weakness in malathion-poisoned chickens appeared to be reversible. This sign disappeared completely within 4 to 21 days in those birds which survived the acute effects of the toxicant.

A few tests with young female chickens weighing about 1 kg. indicated that, although older hens are more resistant to the acute cholinergic effects of malathion, they may be somewhat more susceptible to the muscle weakness.

With EPN, muscle weakness was observed in all of a group of 10 chickens dosed at 60 mg/kg. The minimum dosage giving this effect was 40 mg/kg. In contrast to malathion, the muscle weakness following EPN was irreversible in most cases. Only 3 of the 21 animals which had muscle weakness following the injection of EPN survived. Two of these hens completely recovered from their muscle weakness after 6 and 35 days, respectively. The third survivor still showed marked leg weakness 308 days after dosing. A small number of the hens dosed with EPN showed some degree of recovery of muscle strength and gain in weight about two weeks after dosing, and then later became worse in both respects.

A significant number of the hens given higher dosages of malathion and EPN continued to lose weight for as long as 30 days after dosage. This weight loss appeared unrelated to the leg weakness which occurred in some of the same chickens, because the two symptoms were not always present concurrently.

COMMENT

There has been a good correlation between paralytic effect in the chicken and a similar effect in man. TOCP and Isopestox have produced delayed paralysis in both species. DFP alone of the organic phosphorus compounds causing delayed paralysis in chickens has failed to elicit this syndrome in man. A number of compounds which have failed to produce the disease both in man and in chickens are listed in Tables 1 and 2.

The finding of acute muscle weakness in chickens is of considerable interest because of its possible relationship to the delayed paralytic effect. The relationship may not be the same for all compounds or all species, and it may represent mere coincidence when it does occur. However, it should be noted that the most severely affected chemical worker who developed "ginger paralysis" following Isopestox intoxication showed severe, acute muscle weakness immediately after exposure, from which she recovered prior to onset of the delayed paralytic symptoms.

Parker [10] has reported the illness of a child, which he attributed to malathion. The signs and symptoms which this child developed two days after initial exposure to malathion were somewhat superficially suggestive of the weakness observed in chickens dosed with malathion or EPN by a different route. The fact that tightness in the chest, increased secretion from the respiratory tract, sweating, miosis, and fasciculations of the skeletal muscles were not specifically reported for the child suggests that ordinary poisoning by an organic phosphorus compound was not involved; however, comparable changes do not always appear in chickens which develop leg weakness. The presence of stiffness of the neck, positive Kernig's and Brudzinski's signs, a polymorphonuclear leucocytosis, and especially fever (103 F) led to the conclusion that an infection was the etiological agent. The fact that most of the spinal fluid findings were normal does not detract from this conclusion.

Thus, it should be understood that the paralytic effect of large subcutaneous doses of malathion and EPN in chickens is not interpreted as an indication that a similar effect in man will result from exposure to these insecticides by the more usual routes. However, the finding of this sequela of malathion and EPN exposure in chickens should be a reason for exercising appropriate safety precautions until even more widespread use experience enables one to judge more fully the hazard connected with these compounds under conditions of actual usage. In this connection it must be mentioned that 8,000,000 lb. of malathion and a large amount of EPN have been manufactured and sold since the compounds were introduced for agricultural use in 1952 and 1950, respectively. The toxicity of EPN, as indicated by acute and chronic feeding studies, compares favorably with the toxicity of parathion and TIPP, and on this same basis malathion appears to be among the safest (if not the safest) organic phosphorus insecticides available.

SUMMARY

With use of the paralytic effect in the chicken as the best available index of a possible paralytic effect in man, a group of organic phosphorus insecticides has been screened for this property. The compounds tested include chlorthion, DDVP, demeton, diazinon, EPN, malathion, and OMPA.

No delayed paralytic effects were seen with any of the compounds tested.

The chickens dosed with malathion and EPN developed leg weakness immediately after dosing.

This weakness was reversible after malathion but was essentially irreversible after EPN.

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PUBLICATION

32

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Vol. 15, No. 1, March, 1956

TOXICOLOGY PROGRAM
Technology Branch
Communicable Disease Center
Atlanta 22, Ga.

1363. Hormonal effects on DDT storage in the white rat.

William F. Durham, Cipriano Cueto, Jr. and Wayland J. Hayes, Jr. (introduced by Carl C. Pfeiffer). *Communicable Disease Ctr., Public Health Service, Savannah, Ga.*

Sexual differences in the response of rats to DDT dosage have been reported for chronic toxicity, histopathology, and fat storage of the insecticide and its derivative, DDE. The present study was designed to investigate the physiological basis of the two storage differences. Diethylstilbestrol (DES) and testosterone propionate (TP) in peanut oil solution were administered subcutaneously to intact and gonadectomized rats fed 200 ppm DDT in their diet and to control animals. As expected, TP dosage increased the growth of female rats while DES inhibited the growth of males. Either hormone inhibited gonadal growth in young rats of the opposite sex. Rats receiving either DDT or hormone showed an increased liver weight/body weight ratio as compared to control animals. This effect did not appear to be due to the oil vehicle in which the hormone was dissolved. DES increased DDT and DDE storage in fat in the male while TP decreased these values in the female. Similar effects of hormone dosage were noted on the ratio of DDE to total DDT-derived material stored in the fat. Gonadectomy brought about, in both sexes, changes in DDT and DDE storage which were qualitatively the same as those resulting from hormone dosage, but lesser in magnitude. There was some indication of an additive effect between gonadectomy and hormone dosage on DDT and DDE storage in the female rat but not in the male.

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PESTICIDE
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545

Environmental Protection Agency
Chamblee, Georgia 303

131. *The Action of Pesticides on Conduction in the Rat Superior Cervical Ganglion Preparation In Vitro.* Ernest Whitcomb, Environmental Protection Agency, P.O. Box 490, Perrine, Florida.

The findings of some investigators have suggested that DDT is capable of altering the stability of the nerve fiber. The present experiment was designated to determine if DDT, carbaryl, and chlordane, could alter the propagated action potential in the nerve fiber and at the synapsis using the superior cervical ganglion preparation of the rat. The in vitro recording from nontreated animals had the following characteristics: (1) preganglionic nerve fibers membrane requiring approximately 400 msec for 100% recovery; (2) no evidence of spontaneous preganglionic firing; (3) no synchronized firing of postganglionic fibers via stimulation of the preganglionic nerve. Carbaryl and chlordane alter the control pattern by shortening the recovery time. DDT lengthened the recovery time. The lengthened recovery time and absence of spontaneous firing in preparations from DDT-treated animals would not implicate peripheral nerve components as a source for tremors seen in the intact treated animal.

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TOXICOLOGY & APPLIED PHARMACOLOGY 22(2):315-316, JUNE 1972

PESTICIDE
PUBLICATIONS

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Environmental Protection Agency
Chamblee, Georgia 30341

108. *Light Microscopy and Ultrastructure of Liver of Rats Fed Polychlorinated Biphenyls.*

RENATE D. KIMBROUGH, RALPH E. LINDER, and THOMAS B. GAINES, Toxicology Branch, EPA, Chamblee, Georgia.

Polychlorinated biphenyls (PCB) are widely distributed in the environment. Two PCB were fed to groups of 10 male and 10 female weanling Sherman strain rats in their diet at levels of 0, 20, 100, and 500 ppm Aroclor 1254 and 0, 20, 100, 500, and 1000 ppm Aroclor 1260 for 8 mo. In a preliminary study, 5 of 10 male rats and 8 of 10 female rats fed 1000 ppm Aroclor 1254 for 3 mo died. In the 8-mo study one female and two males fed 500 ppm Aroclor 1254 died. Eight females fed 1000 ppm, two females fed 500 ppm and one female fed 100 ppm of Aroclor 1260 died. All other rats survived. The livers of all rats exposed to the Aroclors weighed more than those of the controls. This difference was significant for all exposed male rats ($p < 0.025$) and for the females fed 500 ppm of either compound. Light microscopic examination of the liver of the exposed groups revealed enlarged hepatocytes with occasional inclusions in the cytoplasm. The cytoplasm of the liver cells was often either foamy or coarsely vacuolated and contained a great deal of lipid. A brown pigment was present in Kupffer cells of a number of livers. Adenofibrosis, particularly at the higher dietary levels, was also observed. Ultrastructural changes included increase in smooth endoplasmic reticulum, concentrically arranged membranes in the cytoplasm which surrounded lipid vacuoles, atypical mitochondria, "moth-eaten" appearance of the cytoplasm, and intracytoplasmic vacuoles with strands of electron-dense material. The area of adenofibrosis showed collagen, fibroblasts, and epithelial cells with granular cytoplasm. The epithelial cells formed rosettes which surrounded cellular debris. Their free surfaces were lined by microvilli. Some of the epithelial cells contained a great deal of mucus and resembled goblet cells.

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542

Environmental Protection Agency
Chamblee, Georgia 30341

28. *Evaluation of Normal and Organophosphate and Carbamate Insecticide Inhibited Blood Cholinesterase Activity Utilizing Dimethylbutylacetate as a Substrate.*

M. F. CRANMER and A. J. PEOPLES, Environmental Protection Agency, Perrine, Florida. (W. F. Durham.)

The availability of an extremely sensitive and precise gas chromatographic method for the analysis of human blood cholinesterase led to the attempt to apply this method to common laboratory animals where analysis of limited blood samples is important. Of particular importance was the comparison of plasma activities, since the plasma of different species vary greatly in esterase profiles. In addition to the need to compare activities obtained with dimethylbutylacetate to those expected with standard methods utilizing acetylcholine was the requirement to compare activities resulting after the administration of an anticholinesterase pesticide. Normal and inhibited blood cholinesterase values are presented for weanling and mature rats, mice, and rabbits and for mature Rhesus and squirrel monkeys. The inhibited samples were analyzed 30 min after the animals were given a po dose of parathion and carbaryl equivalent to ¼ the LD50. The results demonstrate DMBA to be acceptable as a cholinesterase substrate both for the determination of normal and inhibited activities in all species except the mouse, where high levels of nonspecific plasma esterases complicate interpretation.

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TOXICOLOGY & APPLIED PHARMACOLOGY 19(2):416, JUNE 1971

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Environmental Protection Agency
Chamblee, Georgia 30341

(143) *Dermal Phototoxic Reaction from an Insecticide: Clinical and Laboratory Studies.*

W. C. FELSENSTEIN, T. GAINES, and D. C. STAIFF, Food and Drug Administration, Department of Health, Education, and Welfare, Wenatchee, Washington, and Atlanta, Georgia.

Since its introduction into North Central Washington orchards in 1965 application of the cyclic carbonate pesticide Morestan has frequently produced a peculiar skin reaction. Recognition of the influence of weather, involvement of exposed skin surfaces only, and sunburn-like character of the reaction, all suggested a photoactive mechanism. Contact phototoxicity appears confirmed by clinical studies which indicate requirement of presence of the compound on the skin in presence of sunlight, prompt reactivity without the "incubation period" seen in photoallergies, positive photopatch tests, failure of skin protected from contact with Morestan to become sun-sensitive, and protection of susceptible spraymen by application of a sun-screen lotion which forms no mechanical skin barrier. Appropriate analyses revealed no evidence for systemic absorption of Morestan or secondary skin photosensitization via abnormal porphyrin production in exposed individuals. Limited animal studies have produced positive skin reactions in rats. In the laboratory, photoactivity has been demonstrated in vitro with an interesting biological test. Prompt photodecomposition of the compound occurs with unknown reaction product(s) which are under study elsewhere. Evidence was found that under special conditions Morestan can also produce simple irritations and true allergic contact dermatitis.

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PESTICIDE
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by mouth

gamma

112. *Metabolic Alterations in the Squirrel Monkey Induced by DDT Administration and Ascorbic Acid Deficiency.* R. CHADWICK, M. CRANMER, and A. PEOPLES, DHEW, Perrine Primate Research Branch, Box 490, Perrine, Florida. (W. F. Durham.)

In experimental animals, agents which induce drug metabolizing enzymes frequently stimulate the excretion of ascorbic acid via the glucuronic acid system. Ascorbic acid deficiency in the guinea pig, which cannot synthesize the vitamin, has been reported to inhibit hepatic mixed function oxidases. The effects of DDT stress and ascorbic acid deficiency on various mixed function oxidases, enzymes of the glucuronic acid system, and the metabolism of ^{14}C-γHCH and ^{36}Cl-DDT were studied in the squirrel monkey. Daily po injections of 5 mg DDT for a 2-wk period produced significant increases in liver weight, microsomal protein cytochrome P-450, and the in vitro activity of various mixed function oxidases. DDT pretreatment also significantly increased elimination and decreased storage of radioactivity from ^{14}C-γHCH but not from ^{36}Cl-DDT. Ascorbic acid deficiency significantly inhibited the ratio of glucuronic: glucaric acid excreted in 24 hr as well as the increase in the *O*-demethylation of *p*-nitroanisole resulting from the DDT pretreatment. Results from this study suggest that interactions between nutritional status and toxic stress may significantly affect some routes of detoxification in mammals.

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Chamblee, Georgia 30341

111. *Fate of ^{14}C-Labeled o,p′-DDT in the Rat.* M. CRANMER, W. OLLER, J. CARROLL, and F. COPELAND, DHEW, Perrine Primate Research Branch, Box 490, Perrine, Florida. (W. F. Durham.)

The intent of this work was to test the reported observation that *o,p′*-DDT is isomerically converted to *p,p′*-DDT by the rat. *o,p′*-DDT at 50 ppm in the feed was fed to 12 of 24 female Sprague-Dawley rats weighing 200–250 g for 12 wk. Three consecutive days prior to sacrifice, ^{14}C-labeled *o,p′*-DDT was administered to the 12 rats receiving *o,p′*-DDT in their diet and to 6 of the 12 controls. Fat samples were prepared for analysis by the Mills, Olney Gaither method, analyzed by gas–liquid chromatography and confirmed by thin-layer chromatography. Radioactivity of individual residues was determined by trapping from the gas-liquid chromatograph followed by liquid scintillation counting. The levels of *p,p′*-DDT or *p,p′*-DDE was not increased over the control levels in any of the 12 rats receiving 50 ppm *o,p′*-DDT for 12 wk. ^{14}C-labeled *o,p′*-DDT was converted to ^{14}C-labeled *o,p′*-DDD, but not to ^{14}C-labeled *p,p′*-DDT or ^{14}C-labeled *p,p′*-DDE.

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Chamblee, Georgia 30341

intravenous

99. *Fate of ^{14}C-Ring and ^{14}C-Ethyl Labeled Parathion in the Rhesus Monkey.* F. COPELAND, M. CRANMER, J. CARROLL, and W. OLLER, DHEW, Perrine Primate Research Branch, P.O. Box 490, Perrine, Florida.

There is a need for an experimental species which resembles man in the effects produced by exposure to parathion, a widely used insecticide. ^{14}C-Ring- and ^{14}C-ethyl-labeled parathion (0.3 mg/kg) was administered iv to 8 male rhesus monkeys (*Macaca mulatta*). Plasma and RBC-cholinesterase depression, disappearance of parathion from the blood and metabolites in urine were monitored. Cholinesterase activity decreased sharply for the first 30 min with a maximum depression occurring after 6 hr. Comparison of radioisotopic and gas chromatographic determinations of urinary ^{14}C-ring label, ^{14}C-labeled diethylphosphate (DEP), and diethylthiophosphate (DETP) indicated that 75% of the administered ^{14}C-ring-labeled dose was recovered within 6 hr and 90% within 24 hr, as compared to 35% of the administered ^{14}C-ethyl labeled dose in 6 hr and 54% within 24 hr. The ratio of DEP:DETP changed with time but the total ^{14}C-labeled DEP + DETP to ^{14}C-ring label ratio remained constant.

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Chamblee, Georgia 30341

98. ***Alterations in Liver Microsomal Metabolism of Parathion Caused by Chronic Administration of Dilantin, Chloroquine, or DDE.***

J. E. DAVIS, M. F. CRANMER, and A. PEOPLES, DHEW, Perrine Primate Research Branch, P.O. Box 490, Perrine, Florida.

Monkeys and rats were fed dilantin, chloroquine, or DDE in order to assess the effects of these compounds on liver microsomal metabolism of parathion and the inductive mechanisms involved. Dilantin, chloroquine, or a combination of both drugs was fed to male squirrel monkeys for 1 mo and to female rats for 1 wk. DDE was added to the food of female rats for 18 mo. Liver microsomes were isolated and were incubated with parathion and a NADPH generating system. The parathion metabolites produced were analyzed by gas chromatography. Dilantin increased the cytochrome P-450 level and the metabolism of parathion in monkey liver microsomes, but had no significant effect on either of these parameters in rats. Chloroquine treatment of monkeys and rats did not affect the cytochrome P-450 level in, or parathion metabolism by, the liver microsomes of either species. The feeding of dilantin and chloroquine affected neither the cytochrome P-450 level in, nor the parathion metabolism by, rat liver microsomes. DDE administration greatly increased the liver microsomal metabolism of parathion but had no effect on the cytochrome P-450 level.

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Environmental Protection Agency
[illegible] 30341

86. *Effect of Chloroquin on Hepatic Microsomal Enzyme of the Squirrel Monkey.*

M. CRANMER and A. PEOPLES, DHEW, Perrine Primate Research Branch, Box 490, Perrine, Florida.

Chloroquin, a widely used antimalarial drug, has been shown to inhibit bacterial DNA and RNA synthesis. Malarial infections have been shown to produce alterations in host hepatic P-450 content. The present work was done to examine the possible effect on the hepatic microsomal enzymes and P-450 content of squirrel monkeys treated with chloroquin. Four adult male squirrel monkeys were orally administered (2) 5 mg/kg per day and (2) 10 mg/kg per day of chloroquin phosphate for 30 days, and 4 similar animals served as controls. At the time of sacrifice several hepatic microsomal enzymes and P-450 were determined. Chloroquin was found to stimulate the *o*-demethylation of *p*-nitroanisole and the oxidative hydrolysis of EPN but not to increase hepatic P-450. A 30-day 10 mg/kg/day chloroquin pretreatment to 4 adult male squirrel monkeys blocked the stimulation of P-450 by a 3 day 5 mg/kg administration of *p,p'*-DDT and decreased the stimulation of hepatic microsomal enzymes by *p,p'*-DDT.

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TOXICOLOGY & APPLIED PHARMACOLOGY 19(2):375-376, JUNE 1971

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Environmental Protection Agency
Chamblee, Georgia [illegible]

37. *The Oral and Dermal Toxicity of Hexachlorophene in Rats.*

T. B. GAINES and R. D. KIMBROUGH, Public Health Service, Department of Health, Education, and Welfare, Chamblee, Georgia.

The toxicity of hexachlorophene, a widely used fungicidal and bactericidal agent, was studied in Sherman strain rats. The acute oral LD50 for hexachlorophene in peanut oil solution was 56 mg/kg in female rats and 66 mg/kg in males. Adult female rats fed 500 ppm hexachlorophene in the diet for 97 days developed paralysis in the hindquarters; 1 of 10 rats died. Females fed 100 ppm exhibited no signs of poisoning. Examination of the central nervous system (CNS) by light and electron microscopy showed extensive vacuolization of the myelin sheaths resembling spongy degeneration of the white matter in all rats fed 500 ppm. The brains were slightly affected in 8 of 10 rats fed 100 ppm. Reproduction studies were conducted in male and female rats fed 20 or 100 ppm hexachlorophene in the diet continuously from the time the F_0 generation was 4-wk-old through the F_{1a}, F_{1b}, and to weaning of the F_2 generation. No effect on reproduction was observed in rats fed 20 ppm, and the CNS was morphologically normal. In the rats fed 100 ppm the survival rate of the F_1 generations was slightly reduced, the effect being more pronounced, but not statistically significant, in the F_{1b} rats. No definite effect of hexachlorophene was seen in the F_2 generation. Injury to rat skin by stripping with cellophane tape did not appreciably enhance the effect of a single dermal dose of 600 mg/kg of hexachlorophene in 95% ethanol solution (highest dose tested). Two of 10 stripped-skin rats and 1 of 10 rats with intact skin died of hexachlorophene poisoning. A 3% commercial hexachlorophene preparation on the intact skin of female rats at a dose of 48 mg/kg/day for 30 days produced severe ulceration of the treated skin and extensive diffuse spongy degeneration of the white matter of the CNS; similar treatment with 1.5 and 0.75% hexachlorophene solutions (commercial preparation diluted with water) at doses levels of 24 or 12 mg/kg/day, respectively, caused less severe local reaction of the skin.

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Food and Drug Administration
Chamblee, Georgia 30341

TOXICOLOGY AND APPLIED PHARMACOLOGY, 17(1):276, JULY 1970

Dynamics of DDT Storage in the Squirrel Monkey. M. CRANMER, F. COPELAND, and J. CARROLL, Food and Drug Administration, Perrine Primate Research Branch, P.O. Box 490, Perrine, Florida.

It is often desirable to study exogenous chemicals including pesticides and drugs to ascertain a species which stores and metabolizes the material in a manner similar to man. This investigation was undertaken to study the tissue residues of p,p′-DDT and metabolites in the squirrel monkey.

Thirty squirrel monkeys were divided into five groups of six animals each and were dosed daily with 0.0, 0.05, 0.5, 5.0, and 50 mg p,p′-DDT/kg body weight/day. Two animals of each group were sacrificed after 2, 4, or 6 months of treatment. Blood samples were taken every 2 weeks. The levels of p,p′-DDT, p,p′-DDD, and p,p′-DDE were determined for brain, fat, liver, kidney, adrenal, bone marrow, and blood. The activity of liver microsomal enzymes was determined at the time of sacrifice by studying hydrolysis of paranitroanisole and EPN.

The concentrations of p,p′-DDT and metabolites did not plateau in any of the treatment groups. The levels of p,p′-DDT stored in the group receiving 5.0 mg/kg decreased during the last 2 months of the experiment. Liver microsomal enzymes were stimulated by the 5.0 and 0.5 mg/kg/day doses.

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Toxicology & Applied Pharmacology 19(2):397, June 1971

PESTICIDE
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459

Environmental Protection Agency
Chamblee, Georgia 30341

92. *Direct Effect of Paraoxon on Erythrocyte Metabolism as Measured by O_2 Uptake.* J. A. SANTOLUCITO and E. R. WHITCOMB, DHEW, Perrine Primate Research Branch, P.O. Box 490, Perrine, Florida. (W. F. Durham.)

In studying the effects of anticholinesterase compounds, the blood has been considered primarily as an index of exposure; however, there are reports in the literature which suggest that anticholinesterase compounds may have an effect on the erythrocyte itself. This study was undertaken to measure the metabolic O_2 uptake of rabbit erythrocytes and the rate of erythrocyte removal from general circulation after exposure to paraoxon. O_2 uptake was measured with a Gilson Differential Recording Respirometer. ^{51}Chromium-labeled erythrocytes were used to determine removal rate. The control rabbit erythrocyte utilizes O_2 at a rate of $1.5 \pm 0.2 \times 10^{-15}$ l/RBC/hr. Paraoxon blocked the O_2 uptake. Glutathion restored O_2 uptake following paraoxon inhibition. There was no apparent acceleration of removal rate of the paraoxon treated erythrocyte in the general circulation. These results suggested that paraoxon acted on —SH groups in blocking O_2 uptake.

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No. 389

Food and Drug Administration
Chamblee, Georgia 30341

TOXICOLOGY AND APPLIED PHARMACOLOGY, 17(1):289, JULY 1970

gamma

gamma

Comparative Stimulation of γHCH Metabolism by Pretreatment of Rats with γHCH, DDT, and DDT + γHCH. R. W. CHADWICK, M. F. CRANMER, A. J. PEOPLES, Food and Drug Administration, Perrine Primate Research Branch, P.O. Box 490, Perrine, Florida.

Pretreatment of rats with γHCH, DDT, and DDT + γHCH accelerates the metabolism of γHCH. A single oral dose of ^{14}C-γHCH was administered to all animals after a 2-week pretreatment period. The treated rats excreted significantly more radioactivity and stored significantly less ^{14}C-γHCH than the control animals. In addition to significantly larger livers, the treated rats exhibited more liver protein, higher cytochrome P450 content and greater in vitro enzyme activity than the control animals. The self-induction of γHCH metabolism has not previously been reported and could be of major importance in establishing a steady state relationship between exposure and storage of such pesticides by mammals. Both quantitative and qualitative differences in γHCH metabolism were observed between the treated groups of rats.

Results of this study suggest that the mechanism by which γHCH induces its own degradation differs from the mechanism through which DDT stimulates γHCH metabolism.

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3141

PESTICIDES PROGRAM
National Communicable
Disease Center
Atlanta, Ga. 30333

TOXICOLOGY AND APPLIED PHARMACOLOGY, 14(3):631-632, MAY. 1969

The Oral Toxicity of Mirex in Adult and Suckling Rats. T. B. GAINES and R. D. KIMBROUGH, Toxicology Laboratory, Pesticides Program, Food and Drug Administration, Consumer Protection and Environmental Health Service, Public Health Service, U.S. Department of Health, Education, and Welfare, Atlanta, Georgia. (Sponsor: William F. Durham.)

The oral toxicity of the pesticide mirex (dodecachlorooctahydro-1,3,4-metheno-2*H*-cyclobuta[*c,d*]pentalene), which is used in the control of fire ants, was studied in Sherman strain rats. The single-dose LD50 was 365 mg/kg and the 90-dose LD50, 6.0 mg/kg in adult female rats. The chronicity factor, which is the ratio of single-dose LD50 to 90-dose LD50 [Hayes, *Toxicol. Appl. Pharmacol.* 11, 327–355 (1967)] was 60.8. The highest dietary level of mirex tolerated without symptoms of poisoning was 50 ppm (3.1 mg/kg/day). The livers of adult male and female rats fed 25 ppm mirex in the diet for 166 days showed microscopic changes, and were significantly larger than the livers of the control rats ($p < 0.001$ and < 0.025, respectively). Male and female rats fed 25 ppm mirex in the diet were bred to nontreated rats on days 45 and 102 of treatment. Nontreated females bred to the treated males produced normal, healthy litters. In comparison, there was a significant decrease ($p < 0.05$) in the number of offspring born alive to treated females (102 days of dosing) bred to nontreated males, and in the percent of offspring that survived to weaning ($p < 0.025$, 45 days dosing; $p < 0.001$, 102 days). Thirty-three percent of the offspring from the first breeding (45 days) and 46.2% of those from the second breeding (102 days) developed cataracts before weaning. The percent survival of offspring born to treated mothers (fed 25 ppm mirex for 6 weeks) and transferred to nontreated foster mothers at birth was 70.8%, and 1 of 64 survivors developed cataracts. Only 61.0% of the offspring born to nontreated mothers and transferred at birth to treated foster mothers (fed 25 ppm mirex for 6 weeks prior to breeding and during gestation and lactation) survived to weaning, and 39.1% of these developed cataracts. No cataracts developed in offspring born to control rats, and 95.8% survived to weaning when they were transferred to foster mothers at birth.

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Atlanta, Ga. 30333

TOXICOLOGY AND APPLIED PHARMACOLOGY, 14(3):620-621, MAY 1959

Biochemical Effects in the Rabbit of Repeated Administration of DDT, Carbaryl, and Parathion. A. HASSAN and C. CUETO, JR., Pharmacology Section, Pesticides Research Laboratory, Pesticides Program, Food and Drug Administration, Perrine, Florida.

Some biochemical effects of the repeated administration of DDT (5 mg/kg), carbaryl (5 mg/kg) and parathion (0.5 mg/kg) to rabbits were studied during 222 days. No significant changes were found in the blood or urine levels of amino acids. The tubular phosphorus reabsorption remained unaltered. The blood coagulation time was considerably shortened in the treated animals. The urine of treated animals as compared to controls contained high amounts of 5-hydroxy-3-indoleacetic acid (5-HIAA) and 4-hydroxy-3-methoxymandelic acid (VMA). The higher levels of these urinary metabolites are an indication of an increased rate of metabolism of serotonin and the catecholamines, respectively. This increased metabolism is in turn believed to be a consequence of an increased biosynthesis and release of biogenic amines caused by a "stress mechanism" induced by the administered pesticides. The amounts of 5-HIAA and VMA returned to normal 45 days after cessation of treatment.

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Food and Drug Administration
Chamblee, Georgia 30341

TOXICOLOGY AND APPLIED PHARMACOLOGY, 17(1):276, JULY 1970

DDT Concentrations in Blood, Brain, and CSF of Rats After DDT-Induced Convulsions.

GLENN MORRISON, Food and Drug Administration, Perrine Primate Research Branch, Box 490, Perrine, Florida.

This study was undertaken to ascertain the absolute and relative amounts of p,p′-DDT in blood, brain, and cerebral spinal fluid (CSF) at a given time after the oral administration of an intoxicating dose of the compound in order to elucidate the role of CSF in the pathophysiology of DDT intoxication. Although there have been previous reports of blood and brain concentrations of DDT, our study appears to represent the first reported data of DDT in CSF.

Adult male and female rats were orally dosed with 200, 400, or 600 mg/kg of p,p′-DDT. When convulsions occurred, samples of CSF, blood, and brain were collected and assayed for p,p′-DDT. Means and standard deviations of 0.12 ± 0.03, 3.4 ± 1.1, and 16 ± 3 ppm, respectively, were found. Neither the sex of the animal nor the dosage given appeared to affect the convulsion-associated concentrations in these three tissues.

Blood and brain have lipid concentrations of approximately 400 and 8000 times that of CSF. However, in this study the blood and brain DDT concentrations were only 30 and 130 times that found in the CSF. Thus there was more DDT in the CSF than would be anticipated from its lipid content alone. The significance of this finding regarding a possible storage and/or transport role for CSF in the dynamics of DDT intoxication remains wholly speculative.

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Food and Drug Administration
Chamblee, Georgia [illegible]

Studies on the Safety of DDVP for the Disinsection of Commercial Aircraft *

ROBERT F. WITTER, Ph.D.,[1] THOMAS B. GAINES, B.A.[2]
J. GORDON SHORT, M.D.,[3] V. A. SEDLAK, B.S.[4] & D. R. MADDOCK, M.S.[5]

There is a need for a more effective method for the disinsection of intercontinental aircraft. A study was made of the possible toxic hazard associated with a new method of disinsection using DDVP vapour (O,O-dimethyl-2,2-dichlorovinyl phosphate) as the insecticidal agent. In these experiments, men and monkeys were exposed four times over one- or two-hour periods for a total of 4-8 hours to DDVP vapour in a simulated aircraft cabin. The concentration of DDVP was higher and the exposure periods were longer than those planned for use in disinsection. Concentrations up to 0.7 μg per litre of air produced no effect on the cholinesterase of men or monkeys. It was found that a concentration of DDVP of 0.9-3.5 μg per litre of air caused a slight decrease in plasma cholinesterase of the men and the monkeys. At a DDVP concentration of 7.5-17.9 μg per litre, monkeys exhibited a marked drop in red cell and plasma cholinesterase and showed miosis, but no other signs of poisoning.

INTRODUCTION

The development of resistance to DDT and the inherent limitations in the performance of aerosols currently used to disinsect aircraft have made difficult the destruction of insect vectors of disease in intercontinental aircraft. The discovery of a new volatile organic phosphorus compound, O,O-dimethyl-2,2-dichlorovinyl phosphate (DDVP) (Mattson et al., 1955), which is considerably less toxic than parathion to animals (Durham et al., 1957, 1959) but has high insecticidal properties (Mattson et al., 1955), offered possibilities of a new method of control. It was later demonstrated that effective disinsection of aircraft could be accomplished by using DDVP vapour for 30 minutes.[6] Highly effective control of the test insects was usually obtained within the range of 0.15 μg to 0.25 μg of DDVP per litre of air.[7] This paper presents some studies on the possible toxic hazard of this procedure to the passengers and crew members.

DDVP is known to be a direct inhibitor of the enzyme cholinesterase (Durham et al., 1957). Many studies have shown that with moderate continued exposure to other organic phosphorus compounds, the level of blood cholinesterases can decline long before symptoms of poisoning appear (Kay et al., 1952; Sumerford et al., 1953; Hayes et al., 1957; Bruaux, 1957, 1960; Lebrun & Cerf, 1960; Hayes [8]). In fact, the rate of decrease in the level of erythrocyte or plasma cholinesterase can provide an index of the degree of absorption of organic phosphorus insecticides in excess of a tolerated minimum (Sumerford et al., 1953; Hayes et al., 1957). Therefore, experiments were set up to determine the minimal con-

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[6] Maddock, D. R., Sedlak, V. A. & Schoof, H. F. Preliminary tests with DDVP vapor for the disinsection of international aircraft (Unpublished manuscript for the Technical Development Laboratories).

[7] See the article by Schoof et al. on page 623 of this issue.

[8] Hayes, W. J., jr (1956) Summary of investigations, No. 11, p. 4 (Unpublished report from the Technological Development Laboratories).

8

centration of DDVP vapour that would cause a drop in the concentration of blood cholinesterase in volunteers under conditions similar to those to be used in aircraft disinsection. In this way, a threshold value for the range of DDVP concentration that would cause a measurable pharmacological effect could be determined. Some idea of the factor of safety in the use of this insecticide could be obtained by comparison of this level of DDVP with the insecticidal level of this compound. The factor of safety obtained in this manner should be a conservative one, since, as mentioned before, blood cholinesterase depletion can occur without any symptoms of poisoning. For the same reason, it was felt that the volunteers would not be subjected to any hazard in these studies. Monkeys, as well as humans, were used, since the former have body-weights and ratios of surface-area to volume similar to those of small children.

METHODS

The studies of exposure to DDVP vapour were conducted in the simulated aircraft cabin to be described elsewhere.[1] Seven healthy men and eight *Rhesus* monkeys were the subjects.

The men ranged in age from 19 to 44 years and averaged 29 years old. Each man was given a thorough medical examination before and after each of two experiments. In each experiment, one group was exposed for one hour a day for four consecutive days (a total of four hours); a second group was exposed two hours a day for four days (a total of eight hours). In the first experiment, each man was assigned to a particular seat in the simulated aircraft cabin. In the second experiment, the DDVP concentration was higher, and each man was assigned in turn to front, rear, and middle positions in the cabin. After every daily exposure, the eyes of each man were checked for miosis before he left the mock-up. Three blood samples for cholinesterase determinations were drawn from each man before exposure began. Additional samples were taken each morning before the men entered the simulated cabin. Post-exposure samples were taken one and seven days after last exposure. Plasma and red cell cholinesterase were assayed by the method of Michel (1949) on the day the blood samples were taken.

TABLE 1. EFFECTS IN MEN ON BLOOD CHOLINESTERASE LEVELS PRODUCED BY EXPOSURE TO DDVP VAPOUR IN A SIMULATED AIRCRAFT CABIN

Experiment No.	DDVP concentration		Remarks
	Range (μg/l)	Mean (μg/l)	
1	0.26-0.88	0.49	No change in plasma cholinesterase or red cell cholinesterase in 3 men exposed 4 hours or 3 men exposed 8 hours.
2	0.9-3.5	2.1	Slight drop in level of plasma cholinesterase in 2 of 3 men exposed 8 hours; no change in levels of these enzymes in men exposed 4 hours. These changes are of questionable significance.

The eight monkeys used in these studies had not been exposed to organic phosphorus compounds within the past year. The males ranged in weight from 6.5 kg to 11 kg, and the females from 4.7 kg to 6.0 kg. Two monkeys, one male and one female, were used in each of four experiments. Each pair was exposed to DDVP vapour two hours a day for four consecutive days. The monkeys were placed in individual cages in the middle position in the cabin. In experiments 1 and 2 the monkeys were present in the cabin with the volunteers, but in experiments 3 and 4 they were alone in the cabin. Blood for cholinesterase samples was drawn according to the same schedule as that used for the volunteers.

The DDVP vapour was produced by the vaporizer to be described elsewhere.[2] During the exposure period, samples of air were collected at the front, middle, and rear positions in the airplane cabin. These air samples were collected and analysed for DDVP by a method given elsewhere.[3] The concentration of DDVP in the air varied with the position in the cabin.

RESULTS

The results of the studies of the blood cholinesterase activities of men are given in Fig. 1 and 2 and summarized in Table 1, and the results for

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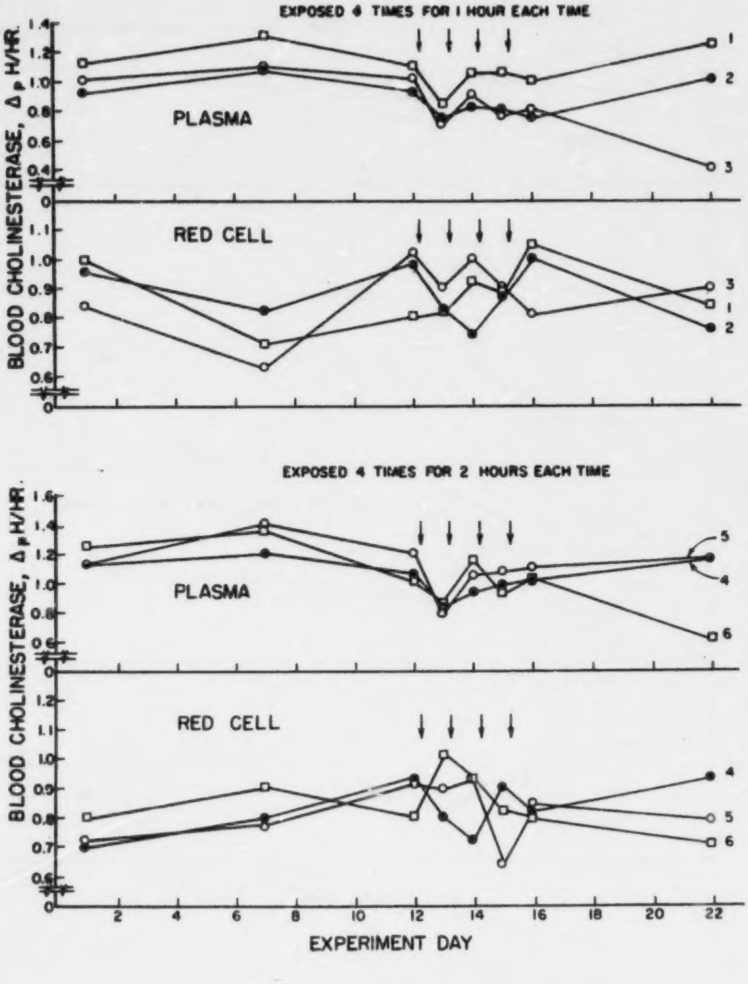
[1] Maddock, D. R., Sedlak, V. A., Schoof, H. F. & Pearce, G. W. Biological effectiveness of vapor deposited residues of DDVP on different aircraft surfaces (Unpublished manuscript from the Technological Development Laboratories).

[2] Maddock, D. R., Sedlak, V. A. & Schoof, H. F. Preliminary tests with DDVP vapor for the disinsection of international aircraft (Unpublished manuscript from the Technical Development Laboratories).

[3] Chemical Memorandum Number 6, 1960. *Determination of DDVP in air* (Unpublished document from the Technical Development Laboratories).

FIG. 1

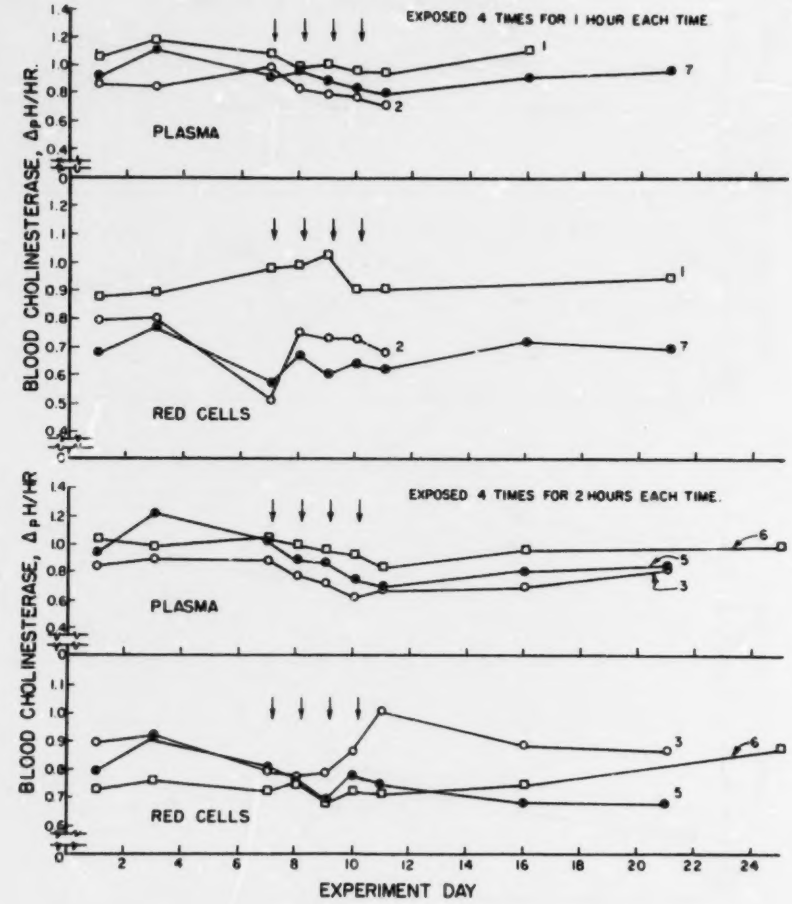
PLASMA AND RED CELL CHOLINESTERASE OF MEN EXPOSED TO DDVP VAPOUR AT CONCENTRATIONS OF 0.26-0.88 μg/l



monkeys are given in Fig. 3-6 and summarized in Table 2. The arrows in Fig. 1-6 indicate the periods of exposure. The odd numbers in Fig. 3-6 relate to the male, and the even numbers to the female monkeys. The levels of DDVP vapour presented in the tables and in the legends for the figures are the ranges for a total of four days of exposure for two hours a day. The DDVP concentration was also determined at the end of one hour. The range and average of concentration of DDVP vapour were essentially the same for the men exposed one hour per day as for the men exposed two hours per day. The range and average of DDVP concentration given for the experiments with men include all the levels found for the front, middle, and back positions in the cabin. Since the monkeys were always in the

FIG. 2

PLASMA AND RED CELL CHOLINESTERASE OF MEN EXPOSED TO DDVP VAPOUR AT CONCENTRATIONS OF 0.9-3.5 μg/l



middle position, the values for DDVP concentration given in Fig. 3-6 and in Table 2 are those obtained for the air in this position in the simulated aircraft cabin.

As shown in Fig. 1, no changes were noted in the cholinesterase levels of the volunteers exposed to DDVP at air concentrations of 0.26 μg to 0.88 μg per litre other than those consistent with normal variation as shown in the pre-exposure samples. When the DDVP concentration was increased to a level ranging from 0.9 μg to 3.5 μg per litre, or an average of 2.1 μg per litre (Fig. 2, No. 6 and 3), a slight drop was noted in the level of plasma cholinesterase of two of the three men exposed four times for two hours each time. The activity of red blood cell cholinesterase in one of the men rose during this same exposure (Fig. 2, No. 6). These results, including the greater sensitivity of plasma cholinesterase as

FIG. 3. PLASMA AND RED CELL CHOLINESTERASE OF MONKEYS EXPOSED FOUR TIMES FOR TWO HOURS EACH TIME TO DDVP VAPOUR AT CONCENTRATIONS OF 0.32-0.66 μg/l

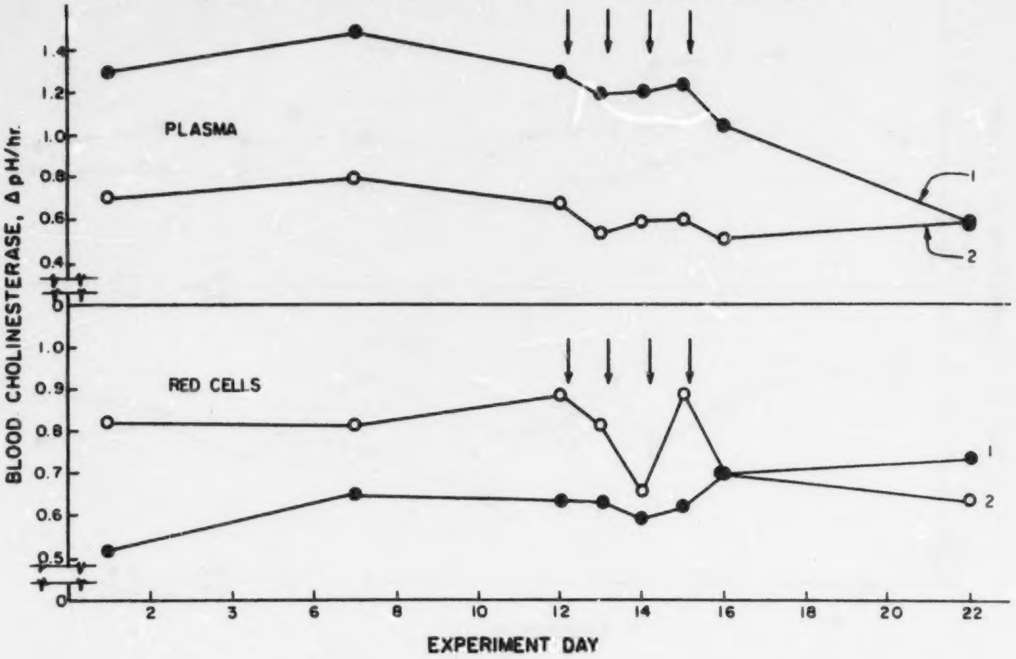


FIG. 4. PLASMA AND RED CELL CHOLINESTERASE OF MONKEYS EXPOSED FOUR TIMES FOR TWO HOURS EACH TIME TO DDVP VAPOUR AT CONCENTRATIONS OF 1.2-3.3 μg/l

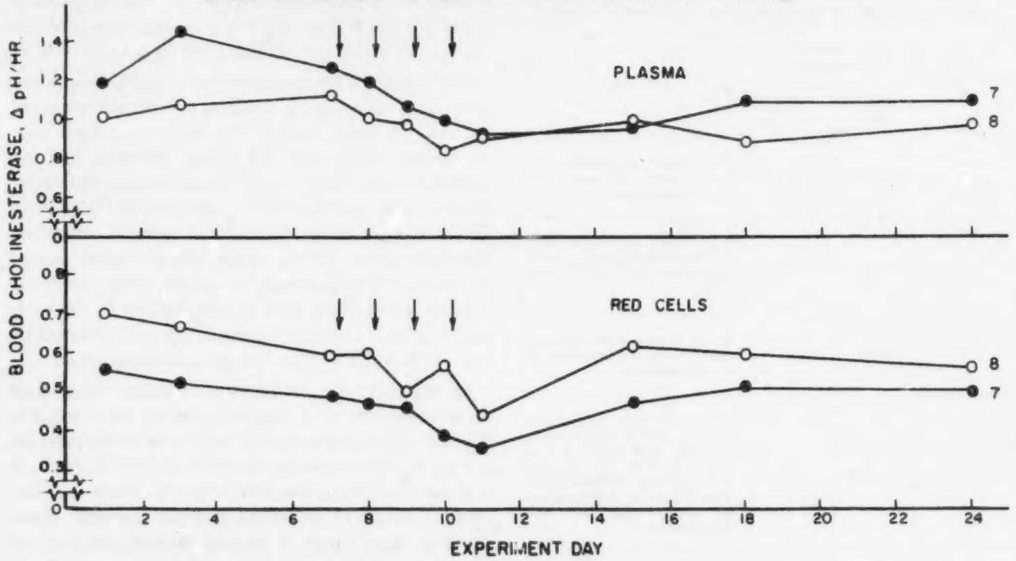


FIG. 5. PLASMA AND RED CELL CHOLINESTERASE OF MONKEYS EXPOSED FOUR TIMES FOR TWO HOURS EACH TIME TO DDVP VAPOUR AT CONCENTRATIONS OF 1.9-3.3 μg/l

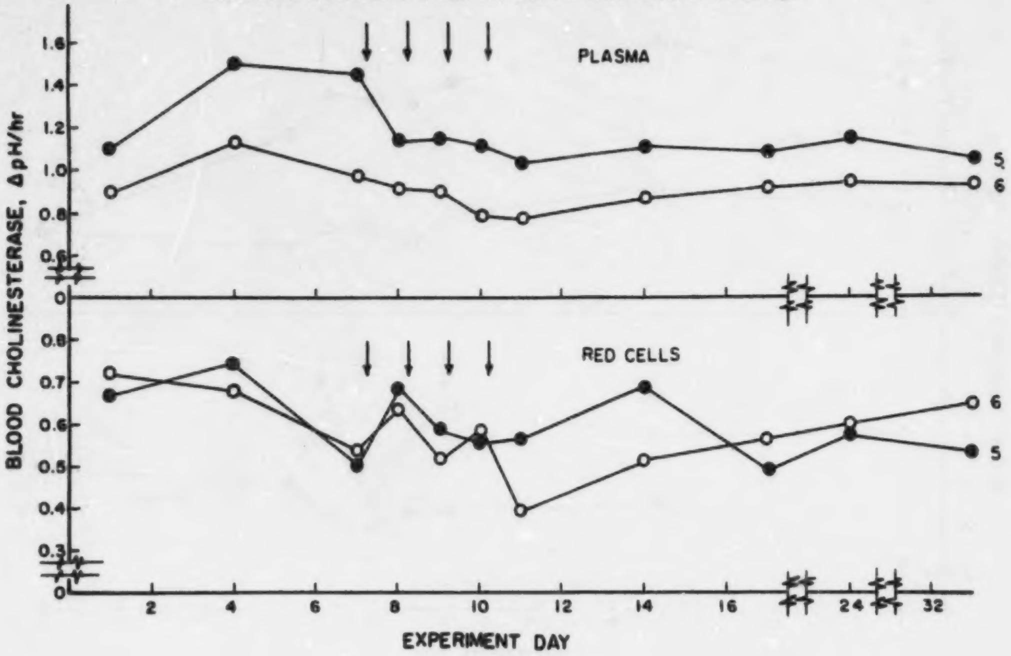


TABLE 2. EFFECTS IN MONKEYS ON BLOOD CHOLINESTERASE LEVELS PRODUCED BY EXPOSURE TO DDVP VAPOUR IN A SIMULATED AIRCRAFT CABIN

Experiment No.	DDVP Concentration		Remarks
	Range (μg/l)	Mean (μg/l)	
1	0.32-0.66	0.48	No change in plasma cholinesterase or red cell cholinesterase.
2	1.2-3.2	2.3	Slight drop of questionable significance in plasma cholinesterase of 1 of 2 monkeys.
3	1.9-3.3	2.6	Slight questionable drop in plasma cholinesterase of 1 of 2 monkeys.
4	7.5-17.9	12.9	Both monkeys tested had miosis, which disappeared between test days. A pronounced drop in red cell and plasma cholinesterase was observed, lasting several weeks after test was discontinued.

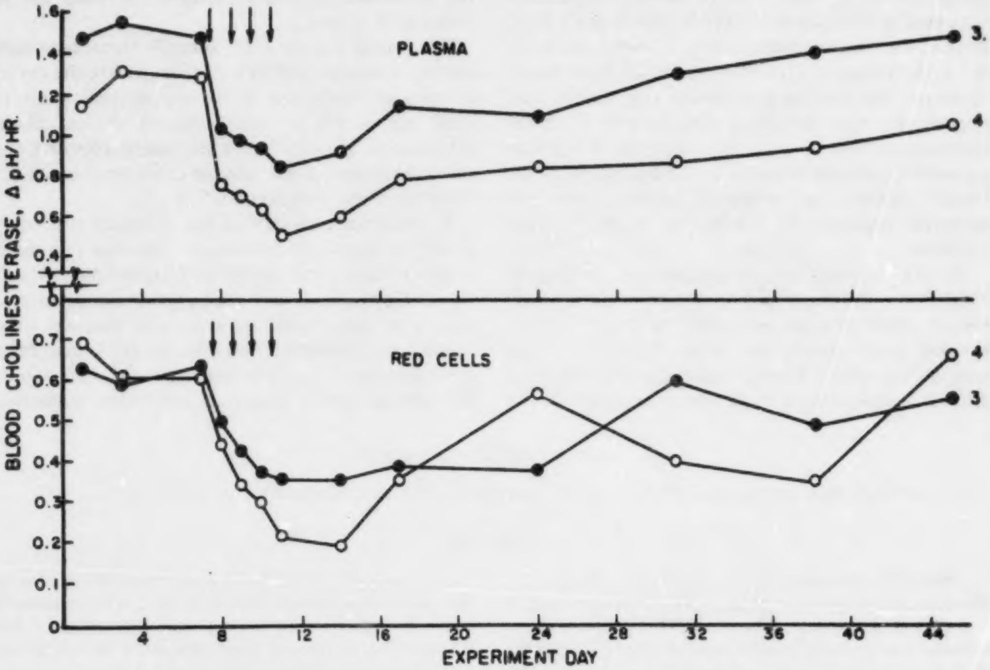
compared with that of red cell cholinesterase, are in accord with previous studies of the respiratory toxicity of DDVP to workers in tobacco warehouses treated with this insecticide (Durham et al., 1959).

The results of the post-exposure medical examination of the men were in agreement with the results of the cholinesterase assays. No cholinergic signs such as miosis were observed, and exposure had no apparent effect on the health of any of the volunteers. Some people working with concentrated DDVP solutions have noted an unpleasant metallic aftertaste (Durham et al., 1957), which was probably caused by dichloroacetaldehyde or some other impurity present in relatively high concentration in the early preparation. This phenomenon was not reported by any of the volunteers in the present experiment.

In regard to the monkeys, no changes other than those consistent with normal variation were noted in the blood cholinesterases of two of them exposed for a total of four two-hour periods at DDVP levels of 0.32 μg to 0.66 μg per litre (Fig. 3). However, one of two monkeys exhibited a slight drop of questionable significance in plasma cholinesterase in an

FIG. 6

PLASMA AND RED CELL CHOLINESTERASE OF MONKEYS EXPOSED FOUR TIMES FOR TWO HOURS EACH TIME TO DDVP VAPOUR AT CONCENTRATIONS OF 7.5-17.9 μg/l



experiment (Fig. 4, No. 7) conducted at an average DDVP level of 2.3 μg per litre with a range of 1.2 μg to 3.3 μg per litre. Another monkey (Fig. 5, No. 6) showed the same reaction at an average level of 2.6 μg per litre with a range of 1.9 μg to 3.3 μg per litre. When the concentration of DDVP vapour was increased to an average of 12.9 μg per litre with a range of 7.5 μg to 17.9 μg per litre, the activities of both the plasma cholinesterase and the red cell cholinesterase dropped abruptly and did not recover for several weeks, as is illustrated in Fig. 6. At this high level of exposure, the monkeys also exhibited pronounced miosis, which disappeared between the two-hour exposure periods. The animals had no other signs of poisoning, and their body-weights were unaffected. There appeared to be no difference between the results with the male and the female monkeys.

These results are consistent with those of previous studies of the respiratory toxicity of DDVP to monkeys. The level of blood cholinesterases in this species was not depressed after 22 days but was definitely lowered after 50 days of exposure in tobacco warehouses, with the air during the exposure period containing an estimated 0.1 μg to 0.5 μg of DDVP per litre (Durham et al., 1959). A depression of blood cholinesterase level was noted after two monkeys were exposed for 20 hours in a Peet-Grady chamber to 2.0 μg to 6.0 μg of DDVP per litre (Durham et al., 1957). The monkeys in these experiments did not exhibit miosis (Durham et al., 1957, 1959).

DISCUSSION

Under the conditions of the tests reported in this paper, the threshold range for a slight effect on blood cholinesterase of man appears to lie in the range of 0.9 μg to 3.5 μg per litre. As indicated above, highly effective control of insects was obtained within the range of 0.15 μg to 0.25 μg of DDVP per litre. The transient enzyme depression found at 0.9 μg to 3.5 μg per litre was the only effect noted in this study. This range of values for the threshold concentration of DDVP is in essential agreement with that of Durham et al. (1959), who found a slight drop in the plasma cholinesterase of men exposed to 0.7 μg to 1.0 μg of DDVP per litre for eight hours. However, the men in the present experiment were exposed for four two-hour periods with 22 hours between exposure periods. This schedule of exposure permitted time for recovery and metabolism of the organic phosphorus compound between doses and probably accounts for the higher threshold range reported.

Previous studies on the respiratory toxicity of DDVP to monkeys, which were carried out in Peet-Grady chambers, showed that the blood cholinesterase levels which had been depleted by continuous exposure to much higher concentrations of DDVP actually began to recover over a period of a week despite the continuous presence of 0.1 μg to 0.2 μg of DDVP per litre (Durham et al., 1959). This concentration is of the order of magnitude of the proposed insecticidal level to be used for 30 minutes at a time.

The rapid recovery of animals from near-fatal acute poisoning by DDVP (Gaines, 1960), the ability of rats to withstand a dietary concentration of 1000 p.p.m. for 90 days without clinical illness (Durham et al., 1957), and the results reported and reviewed above all indicate the capacity of mammals to detoxify the compound efficiently.

It therefore appears that the proposed procedure would be safe to the passengers and crew of a plane if disinsection were carried out according to directions. However, as an added precaution, additional long-term tests including studies of reaction time, visual accommodation and other physiological functions important to pilots and crew members should be made before the procedure can be recommended.

ACKNOWLEDGEMENT

The cholinesterase assays were carried out by Miss Louise McLeod, Histopathology Technician.

RÉSUMÉ

Le DDVP (phosphate de O,O diméthyl dichloro-2,2 divinyl) a été proposé récemment pour la désinsectisation des aéronefs, en remplacement du DDT auquel les insectes vecteurs sont devenus partiellement résistants. La concentration nécessaire et utile est de 0,15-0,25 μg par litre d'air.

Les auteurs ont étudié la toxicité éventuelle de ce produit, en prenant pour critère le taux des cholinestérases sanguines, signe biologique qui précède de beaucoup les premiers symptômes d'intoxication. Ce signe peut être considéré comme l'indice d'un degré d'absorption de composés organophosphorés dépassant le minimum toléré. On a cherché à établir quelle concentration de DDVP provoquait une baisse significative du taux plasmatique ou érythrocytaire des cholinestérases de l'homme et du singe.

A la concentration de 0,9-3,5 μg/l, une légère baisse du taux des cholinestérases fut notée chez 2 des 3 hommes et 2 des 4 singes mis en contact avec l'insecticide 4 fois 2 heures chacun. Aucun signe clinique ne fut noté à cette concentration. En revanche, exposés à des concentrations de 7,5-17,9 μg/l en deux périodes de 4 heures, des singes présentèrent une baisse notable du taux des cholinestérases, et du miosis, mais aucun autre signe d'intoxication.

Il semble donc que l'emploi du DDVP aux concentrations prévues ne fait courir aucun risque aux passagers et à l'équipage des aéronefs. Avant de recommander le procédé, il y a lieu cependant de poursuivre les essais, en vérifiant que l'action à long terme du DDVP, qui pourrait affecter des fonctions physiologiques importantes telles que le temps de réaction et l'accommodation visuelle, est également nulle.

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Cardiovascular Effects of o,p'-DDD*

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Daily oral administration of o,p'-DDD to dogs produced glucocorticoid deficiency. Blood pressure and heart contractile force and rate response to graded doses of epinephrine and norepinephrine were determined in treated and untreated dogs. Vasopressor and cardioaccelerator responses to ouabain were similar in treated and untreated dogs. The induced glucocorticoid deficiency produced little apparent direct circulatory effect but diminished the ability to withstand stressful procedures such as thoracotomy and multiple injections of vasopressor amines.

The adrenal cortical atrophy produced by technical DDD in dogs was first described by Nelson and Woodard in 1948.[1,2] Later, other investigators[3-6] demonstrated that the adrenal atrophy and insufficiency resulted mostly from the contaminant isomer, o,p'-DDD, rather than to the main p,p'-DDD component.

The relatively few detailed controlled studies on the importance of the glucocorticoid hormones in the maintenance of normal circulatory functions have been made in adrenalectomized animals. These animals may be maintained indefinitely with salt and deoxycorticosterone in a protected environment; however, such animals are unable to respond normally to stressful conditions and are more susceptible to cardiovascular failure from traumatic events than normal animals. Therefore, the glucocorticoid hormones appear to be necessary for the maintenance of a normal circulatory state, and these steroids may be required for normal hemodynamic responses following application of various stresses.

Osorio and Kraemer[7] reported in 1958 decreased blood pressure responses to epinephrine, norepinephrine and acetylcholine in dogs with adrenal atrophy produced by technical DDD. No other cardiovascular functions were tested.

Oral administration of the o,p'-isomer of DDD to dogs at a dosage of 50 mg/kg/day for 14 days produces a specific deficiency of 17-hydroxycorticosteriods; however, the adrenal medulla remains intact and normal salt and water metabolism is maintained.

RESULTS AND DISCUSSION

Histological Effects

The histological effects of o,p'-DDD on the adrenal cortex are those characteristically associated with hypophysectomy. However, it has been demonstrated by Brown[8,9] that the pituitary is not involved and that the effect of o,p'-DDD is directly on the adrenal gland. The adrenal cortex of the o,p'-DDD-treated dog as compared to the control animal showed a compression of the reticularis, and a reduction in size of the fasciculata which contained large vacuoles and small nuclei. The glomerulosa was relatively unchanged except for its invagination by fibrous bands which extended from the capsule into the cortex.

Functional Effects

The eosinopenic and plasma 17-OHCS response to adrenocorticotropic hormone (ACTH) was suppressed in the treated dog. There was also a decrease in the basal level of the plasma glucocorticoids. Other measurements (initial blood pressure and heart rate, hematocrit, plasma sodium and potassium, and body weight) were not significantly different from the untreated animals.

The effects of glucocorticoid deficiency produced by o,p'-DDD on the cardiovascular response to catecholamines were then evaluated. The dogs were anesthetized with a pentobarbital-barbital mixture, vagotomized, and the chest opened by a mid-sternum incision. The contractile force response to graded doses of norepinephrine and epinephrine was measured with a strain gauge arch.

Contractile Force Response

There was a significant decrease in the contractile force response to various dosages of epinephrine and norepinephrine in the treated dogs as compared to the controls. The contractile force response decreased more at the higher doses of amines (3.2 mg/kg) than at the lower doses.

Heart Rate Response

The cardioaccelerator response to various dosages of the amines was similar in o,p'-DDD-treated and

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*From the Pesticide Program, Food and Drug Administration, Consumer Protection and Environmental Health Service, U.S. Department of Health, Education and Welfare, Perrine, Florida.

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untreated dogs, but a significant depression of basal heart rate developed in the treated dogs which was not evident prior to surgery.

Diastolic Blood Pressure Response

The diastolic blood pressure response to the amines was similar in both the o,p'-DDD-treated and untreated dogs, but a significantly decreased basal blood pressure developed in the treated animals during the experimental procedure. This was contrary to the findings of Osorio and Kraemer[7] using technical DDD.

Effect of Procedures

An effect of the experimental procedures on the basal diastolic and systolic blood pressure was noted. In the treated but not in the normal animal there was a progressive decline in blood pressure with successive procedures. Oral administration of prednisolone for three days prior to the pharmacological evaluations prevented this hypotensive effect in DDD-treated dogs.

The prednisolone pretreatment was also capable of preventing the effects of glucocorticoid deficiency on the positive inotropic action to the lower doses of amines and to partially, but significantly, prevent the effects of the deficiency on the response to the higher doses.

Histamine and Blood Pressure

The possibility of the release of histamine by the stressful procedures in the glucocorticoid-deficient animal led to the fluorometric analysis of plasma histamine during the progressive hypotensive state. The results obtained demonstrated that histamine is not involved in producing the hypotensive effect.

Effect on Plasma Volume

In another group of dogs it was demonstrated that the development of a progressive hypotensive state was also produced in o,p'-DDD-treated dogs by successive dose response determinations to norepinephrine and epinephrine without the initial stressful event of a mid-sternum thoracotomy. Plasma volume measurements were made in these animals using the Evans blue dilution method. The plasma volume was determined prior to and after each dose response sequence of the amines. Although the plasma volume was initially the same, a significant decrease was detected after the second dose response sequence in the o,p'-DDD-treated dogs but not in the untreated animals nor in the prednisolone plus o,p'-DDD-treated dogs. The decrease in plasma volume of the glucocorticoid-deficient animals occurs with an increased hematocrit but without an alteration of the sodium and potassium concentration in the plasma.

SUMMARY

The administration of o,p'-DDD produces a selective glucocorticoid deficiency in dogs. These animals show a cardioaccelerator and pressor response to catecholamines similar to that of untreated animals, but possess a decreased positive inotropic response to epinephrine and norepinephrine.[10] The vasopressor amines appear to act as stressful agents in these animals precipitating a slowly progressive hypotensive failure. This failure may be due in part to a loss of fluid from the intravascular compartment. The hypotensive failure is not due to the release of histamine.

The decrease in myocardial contractile force and in plasma volume and the development of a progressive hypotensive state in the o,p'-DDD glucocorticoid deficient dog may be significantly prevented by pretreatment with prednisolone.

ACKNOWLEDGEMENT: This study was conducted under the direction of Dr. Neil C. Moran at the Department of Pharmacology, Emory University, Atlanta, Georgia.

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TOXICOLOGY PROGRAM
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Communicable Disease Center
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Relative Toxicity of Insecticides*

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IT is of great importance that we achieve a balanced view of the problem which is before us. This may be done in part by reviewing certain statistics on fatal chemical poisoning. Table 1 reveals that chemical poisoning occupies a minor place as a cause of death. Accidents of a mechanical nature are more common. Table 2 shows that there has been no significant change in the rate of fatal chemical accidents during the last decade. This may be somewhat surprising when one considers the rapid increase in the number and tonnage of toxic compounds which has taken place during this same period. The quantity of a few pesticides produced in the United States is shown in table 3. Table 4 shows that deaths caused by arsenicals and a few of the other older pesticides, chosen for purposes of illustration, continue to occur at about the same rate as they did in 1940 before the introduction of the newer pesticides. This is explained by the fact that the newer toxicants have not so much supplanted as supplemented the older ones.

The relatively good safety record of the older economic poisons is due in part to the fact that workers are aware of their hazard and have learned to treat them with respect. Unfortunately, a part of their reputation for safety depends on a passive acceptance of a small but continuing series of accidents. This same kind of careless thinking may lead one to ignore the importance of common household chemicals and non-prescription drugs as a source of danger. Accidents associated with these materials are much more frequent than those caused by economic poisons. A home accident survey conducted by the Accident Prevention Service, District of Columbia Chapter, American Red Cross, has illustrated this over a period of years. Of 287 cases of fatal and nonfatal chemical poisoning in which a definite cause was listed in 144 instances, six were caused by insecticides and eleven by rodenticides, while at least 22 were ascribed directly to kerosene and 31 to aspirin. Another good illustration of the danger of household chemicals is the study by Rubin and his colleagues[4], who showed that among 250 consecutive admissions for the ingestion of poisons, 21 per cent of the children studied had taken kerosene, while only 9 per cent had taken one of a variety of pesticides.

The facts which have just been mentioned we believe, should be viewed as a challenge to the medical profession and the pesticide industry to reduce these preventable, and therefore unnecessary, accidents. The solution of the problem will, in the long run, be benefited by a balanced perspective. Scare tactics may create a flurry of intense activity and may result in the correction of real abuses. However, there is such a thing as crying "wolf" too often, and those who resort to scare tactics frequently may end by losing the sheep and the shepherd boy as well.

New Pesticide Accidents

A BRIEF review of the accidents associated with the newer pesticides may now be made. Thirteen fatal accidents have been clearly associated with sodium monofluoroacetate (1080) and five other deaths may have been caused by it. There have been at least six nonfatal cases. The established fatalities have varied from one to five per year since 1946. Al-

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* Paper presented at 38th annual meeting of C.S.M.A., Washington, D.C., Dec. 2, 1951

[1] Scientist Director — [2] Senior Surgeon.

[4] Clin. Proc. Children's Hosp. Washington, 5(3): 57-73, February, 1949.

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though figures are not available regarding production or sale of the compound, it is clear that the amount is small as compared with some of the other pesticides, including parathion. Furthermore, it is only because of the unusually strict controls voluntarily imposed by the manufacturer that the record has been kept as good as it is. It is possible that the introduction of warfarin, a relatively safe rodenticide, may lead to a more restricted use of 1080 and a continuation of the decrease in accidents caused by it—a decrease which seems to have been present for at least a year.

ANTU has a good safety record for man in spite of the fact that at least ten children are said to have eaten baits containing the rodenticide[6]. There remains a need for a fast-acting rodenticide that is safe for man and effective against all species of commensal rodents. Although the new slow-acting rodenticides have a good safety record, there remain rodent control problems which are more conveniently solved by a fast-acting rodenticide.

Among the newer insecticides, the organic phosphorus compounds have produced by far the most fatal accidents. Parathion has accounted for eight deaths in the United States, and there was one other in which the part played by parathion could not be evaluated clearly. A large number of nonfatal cases is known, and the majority is probably never reported. However, the very active educational campaign carried out by the manufacturers has done a great deal to bring information to the farmers and orchardists who buy and use the product. This campaign, along with the efforts of private, state, and federal agencies, has apparently reduced the accident rate, although use of parathion has increased. Industry is to be congratulated for its part in promoting safer practices in the use of economic poisons.

Tetraethylpyrophosphate (TEPP) has caused at least nine fatal accidents, and there are two other cases in which the diagnosis is not established. Among the chlorinated hydro-

TABLE 1

Deaths from selected groups of causes and crude death rates per 100,000 population. United States, 1948.[2]

Cause	*Number*	*Rate*
All causes	1,444,337	988.5
Infections and parasitic disease	73,016	50.0
Cancer and other tumors	202,386	138.5
Disease of the circulatory system	505,716	346.1
Chronic poisoning and intoxication	2,550	1.7
Alcoholism	2,433	1.7
Lead Poisoning	46	0.0
All other chronic poisoning	71	0.0
Violent or accidental deaths	123,009	84.2
Accidental deaths	98,001	67.1
Automobile accidents (except collisions with trains or street cars)	29,915	20.5
Accidental absorption of poisonous gas	2,002	1.4
Acute accidental poisoning by solids and liquids	1,436	1.0

[2] U. S. Public Health Service. Vital Statistics of the United States—Pt. I — 1939-48.

TABLE 2

Total accidental deaths in the United States caused by chemicals and rate per 100,000 population.[2]

	Gases		*Solids and Liquids*	
Year	*Number*	*Rate*	*Number*	*Rate*
1939	1,396	1.1	1,371	1.0
1940	1,562	1.2	1,324	1.0
1941	1,464	1.1	1,191	0.9
1942	1,694	1.3	1,193	0.9
1943	2,038	1.5	1,254	0.9
1944	1,907	1.4	1,381	1.0
1945	2,131	1.6	1,532	1.2
1946	1,874	1.3	1,536	1.1
1947	1,938	1.4	1,504	1.0
1948	2,002	1.4	1,436	1.0

[2] U. S. Public Health Service. Vital Statistics of the United States—Pt. I — 1939-48.

TABLE 3

Estimated production of selected pesticides in the United States, expressed in thousands of pounds.[3]

Year	*Calcium Arsenate*	*Lead Arsenate*	*DDT*	*BHC*
1939	41,349	59,569	—	—
1940	—	—	—	—
1941	56,136	75,912	—	—
1942	84,136	63,751	—	—
1943	74,854	73,955	—	—
1944	44,350	90,705	9,626	—
1945	25,644	70,522	33,243	—
1946	35,392	56,667	45,651	—
1947	46,904	31,006	49,600	6,197
1948	27,234	24,630	20,240	18,382
1949	16,006	16,866	37,904	27,937
1950	49,474	—	77,973	57,508

[3] Production statistics based on U.S. Tariff Commission and Bureau of Census figures.

TABLE 4

Number of accidental deaths in the United States for selected causes.[2]

Year	*Arsenic and Its Compounds*	*Nux Vomica and Strychnine*	*Tobacco and Derivatives*	*Creosol Compounds*	*Lye and Potash*	*Barbituric Acid and Derivatives*
1939	109	82	7	31	120	201
1940	73	67	7	32	111	246
1941	70	40	7	29	94	232
1942	71	50	12	25	87	197
1943	85	54	6	20	88	226
1944	77	54	12	17	74	270
1945	58	52	9	13	79	392
1946	63	45	11	19	90	436
1947	48	37	10	7	72	418
1948	63	30	8	8	74	419

[2] U. S. Public Health Service. Vital Statistics of the United States—Pt. I — 1939-48.

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[6] Emlen, J.: *Amer. J. Pub. Health,* 37(6): 721-727, June. 1947.

carbon insecticides, chlordane is known to have killed one factory worker. In a second case, the diagnosis is in doubt.

Some fourteen deaths have been listed as resulting from DDT intoxication. All but two of them involved ingestion and, in most, the action of the solvent in the formulation undoubtedly played an important if not deciding role. Two fatalities ascribed to DDT involved *agranulocytosis* and *periarteritis nodosa*. The diagnosis of DDT poisoning in these instances stands on the weakest of foundations.

It is, of course, recognized that there are usually several nonfatal accidents for every fatal one. The reporting of the fatal ones is much better, however, and they serve as a very good indicator of the seriousness of acute poisoning in general.

Chronic poisoning may, in specific instances, be much more significant than acute poisoning. Chronic intoxication is recognized in our vital statistics. As shown in table 1, approximately two thousand five hundred cases are reported annually. Alcoholism accounts for the greater proportion, while the remainder—about five per cent—includes poisoning by lead and a wide variety of other materials. At the present time, cases of chronic poisoning in man by the newer pesticides have not been confirmed unless one lists in this category certain cases involving occupational exposure to organic phosphorus insecticides. If one may judge from animal experiments, intoxication following repeated exposure to small doses of the newer pesticides would be similar to illness caused by a single large dose. Such a relationship does not hold for all chemicals; for example, chronic and acute lead poisoning may be quite dissimilar. We must, therefore, be on the alert for syndromes characteristic of chronic illness. Furthermore, we must remember that man, in addition to being able to report his symptoms, may show different signs of illness than are shown by laboratory animals.

In this connection, a word of caution is needed. While remaining on the alert for exceptions, we cannot afford to forget the general rules. Any case of human illness ascribed to poisoning should be accompanied by convincing evidence of excessive exposure and by evidence that the signs and symptoms resemble those known to be characteristic. If this cannot be done, a diagnosis of specific intoxication should not be made unless every effort has been made by competent specialists to rule out other causes. Careful attention should be given to the principles of epidemiology. In general, those persons with the greatest exposure to a compound will show the greatest effects—if effects are to be shown. This is true even of those materials, such as beryllium, in which there is good evidence that a sensitization reaction constitutes a significant part of the mode of action.

A word of caution is also in order regarding the expression "chronic toxicity." In simple terms, the expression means prolonged poisoning. It is generally caused by repeated small exposures to a toxicant but, in certain instances, it may be caused by one or a few large doses, as with arsenic. Conversely, small repeated doses may result in acute poisoning. An example is poisoning by small, repeated doses of parathion, in which the cholinesterase level is successively lowered until reaching a point where symptoms of acute poisoning occur.

In conclusion, the newer pesticides have, in comparison with the older ones, a good safety record. This is in large part due to intelligent enforcement of the Federal Insecticide, Rodenticide, and Fungicide Act, and of similar legislation in the various states. Educational campaigns carried on by a great variety of groups, including industry, have helped tremendously to familiarize users with the facts and to establish the custom of reading the labels on economic poisons. The American Medical Association, The U. S. Public Health Service, and others have called the attention of physicians to the symptomatology and treatment of intoxication caused by various pesticides.

Since a great many new pesticidal compounds have been introduced in the last decade, it is obvious that many problems require further scientific study. There is nothing to be gained in the long run, however, by irresponsible statements that nothing is now known of the toxicology of the newer pesticides, or that no legal control of their use exists, or—in the absence of epidemiological proof—that a wide variety of illness from which mankind has suffered for generations is now caused by intoxication by the newer economic poisons.

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Dieldrin Poisoning in Man

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In certain programs, 10–20 percent of spraymen applying dieldrin for the control of disease vectors have been poisoned. Half or more of the reported cases were characterized by epileptiform convulsions. In general, the occurrence of poisoning parallels the intensity and duration of exposure but the factors influencing safety are too little understood. Ordinary, careful medical examination may offer the most sensitive and practical way of detecting preclinical poisoning, thus removing workers from exposure to prevent serious illness.

THERE IS NO DOUBT about the value of dieldrin in the control or even eradication of certain vectorborne diseases. Unfortunately, its use under certain conditions has led to poisoning of some of the workers who received extensive daily exposure for long periods. Information on the epidemiology and clinical course as well as the more recently described preclinical signs of this poisoning is bound to be of interest to those who use dieldrin or contemplate its use in control programs. This review was written because information on dieldrin poisoning has not been publicized widely.

If it were possible, it would be desirable to state the conditions which have led to poisoning in one place and those which have permitted the safe use of dieldrin in another locality. Differences in spray practices and in the personal habits of workers are obvious but not very enlightening. It is easy to find examples of "carelessness" in the groups in which cases of poisoning have occurred, but the same is true among workers who have remained well. Dieldrin has been used under strict supervision in some programs and with little supervision in other programs without producing poisoning. The number of workers involved or the amount of dieldrin really used by individual workers in these programs is frequently so small and the duration of use is so short that no useful information can be gained about the safe use of the compound in a large, continuing program.

It would seem that many reported cases of poisoning were the expected result of flagrant disregard of precautions suggested by the World Health Organization (*1*) or other groups. It appears reasonable to conclude that dieldrin should not be used where lack of water or some other factor makes the practice of cleanliness impossible. If the normal standards of hygiene are low, the use of dieldrin

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should be considered only if extensive supervision of the labor force can be exercised.

There is a need for time-motion studies of workers and measurements of their respiratory and especially their dermal exposures under different conditions.

Because of the need for dieldrin, any research would be a great contribution if it proved the value of some combination of procedures permitting safer use of the compound under practical conditions. As indicated below, an approach which appears hopeful is that associated with the early detection of preclinical illness.

Illness in Venezuelan spraymen caused by repeated exposure to dieldrin was first mentioned by Carrillo in 1954 (*2*). Although dieldrin was at first discounted as the cause of sickness, the true relationship was recognized very soon, according to A. Gabaldon (personal communication, 1954). Poisoning similar to that in Venezuela was also observed in Nigeria (*3*) and in Ecuador, according to information supplied by Lópes da Silva in 1956. In the United States, also, poisoning characterized by convulsions and associated with dieldrin and closely related compounds was observed in industrial workers (*4*).

Later and more complete studies of clinical poisoning have been reported by Blázquez and Bianchini (*5–7*). From the work which has been done, the following conclusions seem clear.

Clinical Illness

1. Under practical conditions of work in Venezuela, Ecuador, and Nigeria, clinical poisoning has been reported in about 10 to 20 percent of spraymen (table 1).

2. The earliest observed poisoning occurred after a little less than 3 months of exposure. However, in the most comprehensive study available (7), no cases occurred in less than 4 months of exposure, and only 2 percent of the spraymen exposed less than 8 months became sick. In general, the proportion of sprayers poisoned increased with the duration of their work (table 2). Twenty-six percent of workers exposed 8 months or more became sick. In consideration of the inevitable variation in the intensity of exposure of different workers and also the small number of workers studied with exposure greater than 2 years, the available data offer no indication of variation in individual susceptibility to poisoning although this possibility is not ruled out.

3. All of the reported cases were severe enough for the patients to seek medical aid. Half or more of the patients had convulsions. Although investigators have understandably reserved judgment in some individual cases, the epidemiology of the disease leaves no doubt that most, if not all, of the reported cases represent poisoning. In fact, it is most likely that some cases of real poisoning, especially mild ones, have been misdiagnosed and thus not reported.

4. Mild clinical illness caused by dieldrin poisoning is characterized by the following symptoms: headache (which is frequently persistent and not responsive to drugs), blurred vision, dizziness, slight involuntary muscular movements, sweating, difficulty in sleeping and bad dreams, nausea, and general malaise. (It

Table 1. Dieldrin poisoning cases in three countries

Country	Number of spraymen	Clinical cases	Cases with convulsions	Fatalities	Percent of sprayers poisoned
Venezuela	285	51	22	0	18
Ecuador	92	8	[1] 4	1	9
Nigeria	40	[2] 4	[2] 4	0	10

[1] Possibly underestimated.

[2] One patient had a history of epilepsy.

NOTE: Venezuela—1.25 percent suspension is applied to porous surfaces and a 2.50 percent emulsion is applied to nonabsorbent surfaces at a rate of 1 gm./m.^{2} in each instance. Ecuador—2.50 percent formulations are used at a rate of 0.5 gm./m^2. Nigeria—0.68 percent suspension and emulsion and 1.37 percent suspension are used at rates of 0.27 and 0.54 gm./m^2.

Table 2. Relation of poisoning to duration of exposure to dieldrin, Venezuela[1]

Months of exposure	Number of spraymen with stated months of exposure	Number of spraymen at hazard	Cases of poisoning	
			Number	Percent of those at hazard
0–3.9	69	285	0	0
4–7.9	38	216	5	2
8–11.9	26	178	9	5
12–15.9	54	152	14	9
16–19.9	41	98	13	13
20–23.9	45	57	4	7
24–27.9	7	12	6	50
28–31.9	1	5	0	0
32–35.9	0	4	0	0
36–39.9	4	4	0	0
0–39.9	285	285	51	18

[1] Modified from Blásquez and Bianchini (7).

is entirely possible that other syndromes exist. The poorly defined illness of the European superintendent described by Haworth (*3*) may well be a case in point.)

5. More severe illness is characterized by the symptoms already mentioned and also by stronger jerking of entire muscle groups leading to movement of some part of the body or the limbs or even causing the patient to fall. In extreme cases these movements are accompanied by momentary loss of consciousness.

6. The most severe, nonfatal poisoning observed so far has been characterized by one or more epileptic convulsions with loss of consciousness but without involuntary incontinence of feces or urine. One patient had more than 30 convulsions. Because patients are unconscious during seizures it is likely that some seizures have been overlooked.

7. The circumstances associated with the death of a sprayman in Ecuador are not known except that he was exposed to dieldrin and his illness was characterized by convulsions. (Animal experiments have revealed a form of dieldrin poisoning which, in the absence of treatment, is uniformly fatal. It is marked by convulsions, complete food refusal, and rapid loss of weight. Such poisoning would almost certainly occur in man under certain conditions but has apparently not yet been described.)

8. The convulsions and sudden falls associated with severe illness are of brief duration. Aside from these attacks and the wounds they may produce, signs of illness are not prominent even soon after a convulsion. However, by very careful observation, the following signs may be discovered in many cases of clinical poisoning: slight alteration of reflexes, incoordination (Romberg and other tests), nystagmus, tremor, sweating, dermatographia, and muscular fibrillations (which can sometimes be elicited by striking the muscle or, apparently, by hyperventilation). Occasionally, patients exhibit disorientation or change of personality. Tachycardia and arrhythmia are fairly common.

9. Following removal from exposure, all survivors showed initial improvement. However one patient suffered a recurrence of convulsions 84 days after his last exposure to dieldrin. Some other patients have required as much as 105 days for recovery. In view of these facts and the fact that it has been impossible to follow all cases, it is clear that dieldrin poisoning in man tends to be chronic, but the full extent of the chronicity is not yet known.

10. There is a broad relationship between intensity and duration of exposure, sickness, and the amount of dieldrin in the blood as determined by bioassay. However, the bioassay, as it has been used so far (*6–8*), shows so much individual variation that it is of limited diagnostic value (table 3).

11. Electroencephalograms were abnormal in about half of the clinical cases studied by this method. But the absence of detectable abnormality in many cases, the small degree of abnormality in some other cases, the presence of abnormality in a few unexposed people, and the expense and difficulty of electroencephalography make it of limited value. Detailed information in the tracings has been given by Ducharne (*9*).

Cases clearly involving the ingestion of single doses of dieldrin have not been reported, but such cases have been published in connection with related compounds. Poisoning by aldrin in combination with a solvent was complicated by effects on the liver and especially on the kidneys as well as the nervous system (*10*, *11*). Poisoning of more than 59 persons by endrin eaten as a contaminant of bread involved the gastrointestinal system as well as the nervous system (*12*). Clinical recovery following a single dose of these compounds has been prompt, although the electroencephalogram did not return to a normal reading for almost 5 months in the aldrin case. Similar results would be expected if dieldrin were eaten.

Preclinical Poisoning

An effort to detect preclinical poisoning has been reported by Winthrop and Felice (*13*). A total of 109 spraymen (about half of those employed in Venezuela at the time) were examined. Seventy-two of the 109 spraymen had been exposed to dieldrin for an average of 70 weeks and were currently exposed at the time of examination. Twenty-six of the 109 had been exposed for an average of 30 weeks but had had no contact with the compound for at least 10 weeks prior to the date of examination. In addition to the spraymen, 64 men without occupational exposure to dieldrin but otherwise as nearly comparable to the spraymen as possible were examined as controls.

The following symptoms and signs occurred more frequently in spraymen exposed to dieldrin for an average of 30 or more weeks than in controls: headache, blurred vision, diplopia, tinnitus, dizziness, slight involuntary muscular movements, sweating, difficulty in sleeping and bad dreams, nausea, alteration of reflexes, incoordination, nystagmus, muscular fibrillations, and change in personality.

The parallelism of these symptoms and signs and those associated with clinical illness is remarkable and probably not accidental. It is important to remember, however, that the relationship has not been evaluated either by a statistical analysis of the data collected by Winthrop and Felice or by a practical test. A practical test would consist of an effort to prevent all poisoning severe enough to cause a workman to seek medical care. Serious poisoning could be prevented theoretically if workers were removed completely from dieldrin exposure as soon as they reached some critical level of preclinical effect.

Table 3. Bioassay index for various groups of workers in Venezuela[1]

Group	Range	Mean
Controls (*5*)	[2] 0	0
7 workers exposed 76 days without clinical illness (*5*)	0–36	16
9 workers with grade I intoxication (*5*)	0–90	53
10 workers with grade I intoxication (*6*)	22–100	69
7 workers with grade III intoxication (*5*)	0–100	71
16 workers with grade III intoxication (*6*)	8–100	70

[1] Recalculated from data of Blázques and Bianchini (*6*, *7*).

[2] Occasionally the death from natural causes of insects used in the tests will produce an index greater than zero.

The "symptom profile," the "sign profile," and the resulting "profile index" (*13*) are devices for expressing results conveniently and they deserve serious consideration. However, these devices do not test the data. To be of use they must be based on careful examination of the patient. The profile index should be used or modified as convenience and experience dictate. Not necessarily related to the use of this index is the suggestion that technicians could be trained to administer systems reviews and even perform simple neurological examinations. Records obtained by technicians could be interpreted by a physician who would examine thoroughly those spraymen showing progressive or sufficiently serious abnormalities.

About one-third of the spraymen who were not sick showed some abnormality in their electroencephalograms (*9*, *13*). It is of interest that abnormality of the brain waves may continue for more than 4 weeks after the last exposure to dieldrin. However, for reasons outlined above, the test is of limited value. The usefulness of spinal fluid examination in the diagnosis of dieldrin poisoning is not established although the findings justify further study in laboratory animals.

Considerable study has failed to show any value of the following tests in connection with dieldrin poisoning: urinalysis, complete blood study, chest X-ray, determination of visual fields, psychometric tests, or chemical determination of dieldrin in the blood. Dieldrin can be successfully analyzed after addition to blood in the laboratory (*14*). It may be that the difficulty of chemically analyzing dieldrin in the blood of patients lies in the problem of extraction and purification of the sample. Such problems are well known in connection with the analysis of DDT in blood in contrast to the analysis of DDT in fat.

Prevention

Many of the papers mentioned above have ended with a list of recommendations regarding spray practices. The recommendations have generally been reasonable. Many of the authors stated fully, that dieldrin can be used safely in an insect control program if the recommendations are carried out. Perhaps this would be true if the recommendations were followed in practice. However, it is necessary to face the fact that (except by discontinuing the regular use of dieldrin) poisoning has not yet been eliminated in connection with any insect control program which gave rise to dieldrin poisoning in the first place.

The limitations of present knowledge of dieldrin poisoning and failure to eliminate such poisoning must be kept in mind. It is equally important to remember that (although complete proof is not yet available) there is evidence that ordinary, careful medical examination by a physician (especially systems review and neurological examination) offers the most sensitive and most practical way of detecting preclinical poisoning. If preclinical poisoning is detected early and affected workers are removed permanently from dieldrin exposure, it may be possible that clinical poisoning can be prevented to a large extent. Furthermore, past failure does not eliminate the possibility that the protection of spraymen and, especially, the uncompromising education of those workers in personal hygiene will greatly reduce the incidence of dieldrin poisoning.

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UN ESTUDIO SOBRE LA INTOXICACION PRODUCIDA EN EL HOMBRE POR EL DIELDRIN*

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No cabe duda sobre el valor del dieldrín en el control y hasta en la erradicación de ciertas enfermedades trasmitidas por vectores. Por desgracia, su empleo en determinadas condiciones condujo a la intoxicación de algunos de los trabajadores expuestos a él, cotidiana e intensamente, por largos períodos. Los datos sobre la epidemiología y el curso clínico de estas intoxicaciones, así como los signos preclínicos descritos en fecha más reciente, están llamados a interesar a cuantos emplean dieldrín o piensan emplearlo en los programas de control. Esta revista del asunto se hizo porque los informes sobre las intoxicaciones por dieldrín no han sido suficientemente divulgados.

De ser posible, convendría indicar las condiciones que condujeron a la intoxicación de los trabajadores en una localidad, y aquellas que permitieron el empleo de este insecticida, sin efectos nocivos para aquéllos, en otra. No cabe duda que hubo diferencias en cuanto al procedimiento de su aplicación y a los hábitos personales de los trabajadores, pero de ellas se pueden sacar pocas enseñanzas. Es fácil encontrar ejemplos de "falta de precauciones" en grupos en que se produjeron casos de intoxicación, pero lo mismo se aplica a grupos en los que no se presentaron intoxicaciones. En algunos programas se utilizó dieldrín bajo una supervisión muy estricta y en otros bajo casi ninguna supervisión y en ninguno de los dos casos hubo intoxicación. El número de trabajadores utilizados, o la cantidad del insecticida realmente empleada por ellos en estos programas, son con frecuencia tan pequeños, y la duración de la exposición tan breve, que no es posible allegar datos de utilidad para el uso sin riesgos del compuesto en un programa continuo y duradero.

Pareciera que muchos de los casos de intoxicación notificados fueron la prevista consecuencia de una flagrante desobediencia de las normas propuestas por la OMS (1) o por otros entendidos en la materia. Parece pertinente concluir que el dieldrín no debe utilizarse donde la falta de agua o cualquier otro factor no permita el grado de limpieza requerido. Si las normas regulares de aseo personal dejan que desear, sólo se puede pensar en el uso del dieldrín bajo una estricta supervisión del personal que lo aplica.

Es necesario hacer estudios del cociente tiempo-movimiento de los trabajadores, y determinaciones de su exposición, por vía respiratoria y sobre todo por vía dérmica, bajo diferentes condiciones.

Dadas las aplicaciones del dieldrín en el terreno de la salud pública, toda investigación que condujera a una combinación de medidas de precaución que hiciese posible su empleo en condiciones normales sin consecuencias peligrosas para el personal que lo maneja y aplica, constituiría una contribución valiosa. Como se indica más adelante, una manera de abordar la cuestión que promete buenos resultados en este sentido, va ligada al pronto descubrimiento de los signos preclínicos de la enfermedad.

Carrillo en 1954 (2) fue el primero en mencionar casos de enfermedad, causada por la repetida exposición al dieldrín, entre rociadores de Venezuela. Según Gabaldon (comunicación personal, 1954) al principio se descartó el dieldrín como causa de la intoxicación, pero muy pronto se determinó la verdadera relación. Una intoxicación semejante a la de Venezuela se observó también en Nigeria (1) y en el Ecuador, según información recibida da Lópes da Silva en 1956. En los Estados Unidos se observaron en trabajadores de la industria casos de intoxicación caracterizada por convulsiones, asociada al dieldrín y otros compuestos afines (3).

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* Informe presentado por el autor a la Oficina Sanitaria Panamericana después de su participación, como consultor técnico de la misma, en el Simposio de Investigaciones Toxicológicas del Dieldrín, organizado por la División de Malariología del Ministerio de Salud y Asistencia Social de Venezuela, y celebrado en Maracay, Aragua, Venezuela, el 15 y 16 de mayo de 1957. Se publica en inglés en el *Public Health Reports* de diciembre de 1957.

Blázquez y Bianchini (4–6) han dado cuenta de estudios posteriores y más completos relativos a la intoxicación clínica. De todas las investigaciones realizadas, parecen deducirse las siguientes conclusiones.

Intoxicación clínica:

1. En el empleo práctico del dieldrín en Venezuela, Ecuador y Nigeria se ha notificado intoxicación clínica aproximadamente en una proporción del 10 al 20% de los rociadores (véase el cuadro No. 1).

2. El caso más temprano de intoxicación se observó poco antes de los tres meses de exposición. Sin embargo, en el estudio más completo que hasta ahora se ha realizado (6), no ocurrieron casos antes de los cuatro meses de exposición, y solamente el 2% de los rociadores expuestos durante un período inferior a 8 meses sufrieron intoxicación. En general, la proporción de rociadores intoxicados aumentó con la duración de su trabajo (véase el cuadro No. 2). Teniendo en cuenta la inevitable variación en la intensidad de la exposición de diferentes trabajadores así como el pequeño número de rociadores estudiados cuya exposición excedía de dos años, los datos de que se dispone no ofrecen indicación alguna de variación en la susceptibilidad individual a la intoxicación, aunque no se excluye esta posibilidad.

3. Todos los casos notificados eran de suficiente gravedad para que el enfermo recurriera al médico. La mitad o más de los pacientes sufrieron convulsiones. Aunque, como es natural, los investigadores se han reservado la opinión en algunos casos individuales, la epidemiología de la enfermedad no deja lugar a dudas de que la mayoría de los casos notificados, si no todos, sufrieron intoxicación. En efecto, es muy probable que algunos casos de verdadera intoxicación, especialmente los de forma leve, se

Cuadro No. 1.—*Casos de intoxicación por dieldrín en tres países.*

País	Número de rociadores	Casos clínicos	Casos con convulsiones	Casos mortales	Porcentaje de rociadores intoxicados
Venezuela	285	51	22	0	18
Ecuador	92	8	4*	1	9
Nigeria	40	4†	4†	0	10

* Es posible que hubiera mayor número de casos.

† Un paciente tenía antecedentes epilépticos.

Nota: *Venezuela*—A las superficies porosas se aplica una suspensión al 1,25%, en tanto que a las superficies no absorbentes se aplica una emulsión al 2,50%, a razón de 1 $g./m.^2$ en cada caso.

Ecuador—Se aplican fórmulas al 2,50%, a razón de 0,3 $g./m.^2$

Nigeria—Se emplean suspensiones y emulsiones al 0,68%, y suspensiones al 1,37%, a razón de 0,27 y 0,54 $g./m.^2$ respectivamente.

Cuadro No. 2.—*Relación entre la intoxicación y la exposición al dieldrín.**

Meses de exposición	Número de rociadores		Casos de intoxicación	
	Con determinado número de meses de exposición	Escogidos al azar	Número	Porcentaje entre los escogidos al azar
0–3,9	69	285	0	0
4–7,9	38	216	5	2
8–11,9	26	178	9	5
12–15,9	54	152	14	9
16–19,9	41	98	13	13
20–23,9	45	57	4	7
24–27,9	7	12	6	50
28–31,9	1	5	0	0
32–35,9	0	4	—	—
36–39,9	4	4	0	0
0–39,9	285	285	51	18

* Modificado de Blázquez y Bianchini (6).

diagnosticaran erróneamente y, por consiguiente, no se hayan notificado.

4. La intoxicación clínica leve producida por dieldrín se caracteriza por los siguientes síntomas: cefalalgia (que muchas veces es persistente y no responde a las drogas), visión borrosa, mareos, ligeros movimientos musculares involuntarios, sudación, dificultad para dormir, pesadillas, náuseas y malestar general. (Es muy posible que existan otros síndromes. El mal definido caso del superintendente europeo que describe Haworth (1) puede ser muy bien un ejemplo de ello).

5. Las formas más severas de intoxicación se caracterizan por los síntomas antes citados y, además, por tremores más fuertes de grupos musculares completos que producen movimientos de alguna parte del cuerpo o de las extremidades y hasta pueden causar la caída del enfermo. En los casos agudos, esos movimientos van acompañados de pérdida momentánea del conocimiento.

6. Los casós más graves de intoxicación, no mortal, observados hasta ahora, se caracterizaron por una o más convulsiones epilépticas, con pérdida del conocimiento pero sin incontinencia involuntaria de las heces o la orina. Un enfermo tuvo más de 30 convulsiones. Es posible que, debido a que los pacientes pierden el conocimiento durante los ataques, se omitieran algunas de estas convulsiones.

7. No se conocen las circunstancias relacionadas con la muerte de un rociador en Ecuador, salvo que estuvo expuesto al dieldrín y que su enfermedad se caracterizó por convulsiones. (Los experimentos realizados en animales han revelado una forma de intoxicación por dieldrín que, en ausencia de tratamiento, resulta siempre mortal. Se caracteriza por convulsiones, inapetencia absoluta y rápida pérdida de peso. Es casi seguro que, en determinadas circunstancias, esa forma de intoxicación ocurriría en el hombre, pero, al parecer, hasta ahora no se ha notificado).

8. Las convulsiones y caídas repentinas asociadas a la intoxicación severa son de breve duración. Aparte de estos ataques y de las heridas que produzcan, los signos de la enfermedad no son prominentes, incluso poco después de una convulsión. Sin embargo, en muchos casos de intoxicación crónica se pueden descubrir, mediante una observación cuidadosa, los siguientes signos: ligera alteración de los reflejos, incoordinación, (Romberg y otras pruebas) nistagmo, temblores, sudación, dermatografismo y temblores fibrilares musculares (que algunas veces se pueden inducir por percusión del músculo o, al parecer, por hiperventilación). A veces el paciente muestra desorientación o cambio de personalidad. La taquicardia y la arritmia son bastantes comunes.

9. Al cesar la exposición, todos los sobrevivientes mostraron mejoría inicial. Sin embargo, un paciente sufrió de nuevo convulsiones 84 días después de su última exposición al dieldrín. En algunos otros casos, la convalecencia de los enfermos se prolongó hasta 105 días. En vista de estas experiencias, y a pesar de que ha sido imposible proseguir la observación de todos los casos, es evidente que la intoxicación por dieldrín en el hombre tiende a ser crónica, pero todavía se desconoce la duración de la cronicidad.

10. Existe una notable relación entre la exposición, la intoxicación y la concentración de dieldrín en la sangre, determinado por bioensayo. Sin embargo, el bioensayo en la forma realizada hasta ahora (4, 7) muestra tanta variación en distintos individuos que resulta de valor limitado para el diagnóstico (véase el cuadro No. 3).

11. Los electroencefalogramas resultaron anormales aproximadamente en la mitad de los casos clínicos estudiados con este método. Pero la ausencia de anormalidades perceptibles en muchos casos, el reducido grado de anormalidad en algunos otros, la presencia de ésta en algunas personas no expuestas y el costo y las dificultades de la prueba, hacen que ésta tenga un valor limitado. Ponce Ducharne (8) ha facilitado información detallada sobre los trazados de los electroencefalogramas.

No se han notificado casos concretos de

Cuadro No. 3.—*Indice de bioensayos en varios grupos de trabajadores en Venezuela.**

Grupo	Alcance	Indice
Testigos (5)	0†	0
5 trabajadores expuestos durante 76 días, sin enfermedad clínica (5)	0–36	16
9 trabajadores con intoxicación de grado I (5)	0–90	53
10 trabajadores con intoxicación de grado I (6)	22–100	69
7 trabajadores con intoxicación de grado III (5)	0–100	71
16 trabajadores con intoxicación de grado III (6)	8–100	70

* Calculado de nuevo de los datos de Blázquez y Bianchini (5, 6).

† A veces la muerte de los insectos de experimentación, debida a causas naturales, produce un índice superior a cero.

intoxicación por ingestión de una sola dosis de dieldrín, pero se han descrito casos de este tipo en relación con compuestos afines. La intoxicación por aldrín en combinación con un solvente se complicó por los efectos producidos en el hígado y, especialmente, en los riñones, así como en el sistema nervioso (9, 10). La intoxicación de más de 59 personas por haber ingerido endrín que había contaminado el pan, afectó al sistema gastrointestinal, así como al sistema nervioso de dichas personas (11).

El restablecimiento clínico después de haber ingerido una sola dosis de estos compuestos ha sido rápido, aunque en el caso del aldrín tuvieron que transcurrir casi cinco meses para que los electroencefalogramas volvieran a resultar normales. Cabe suponer que se registrarían resultados similares en el caso de ingestión de dieldrín.

Winthrop y Felice (véase la pág. 512) han informado sobre un estudio que realizaron para descubrir la intoxicación preclínica. Examinaron a un total de 109 rociadores (aproximadamente la mitad de los empleados en Venezuela en aquella época). Setenta y dos de los 109 rociadores habían estado expuestos al dieldrín durante un promedio de 70 semanas y estaban también expuestos en la época en que se hizo el examen. Veintiséis de los 109 individuos habían estado expuestos durante un promedio de 30 semanas, pero no habían tenido contacto con el compuesto por lo menos en las 10 semanas anteriores a la fecha del examen. Además de los rociadores, sometieron a examen, como testigos, a 64 individuos que no habían estado expuestos al dieldrín, por razón de su trabajo, pero que reunían condiciones comparables, en la medida posible, a las de aquéllos.

Los siguientes síntomas y signos se observaron más frecuentemente en los rociadores expuestos al dieldrín durante 30 o más semanas que en los testigos: cefalalgia, visión borrosa, diplopia, tinnitus, mareos, ligeros movimientos musculares involuntarios, sudación, dificultad para dormir y pesadillas, náuseas, alteración de los reflejos, incoordinación, nistagmo, temblores fibrilares musculares y cambio de personalidad.

El paralelismo entre esos síntomas y signos y los asociados con la enfermedad clínica, es notable y probablemente no se debe a causas accidentales. Es conveniente recordar, sin embargo, que la relación no se ha evaluado por un análisis estadístico de los datos recopilados por Winthrop y Felice ni por una prueba práctica. La realización de una prueba práctica supondría un esfuerzo para impedir toda intoxicación suficientemente severa para que el trabajador recurriera al médico. La intoxicación grave se podría prevenir teóricamente si se alejara completamente a los trabajadores de la exposición al dieldrín tan pronto como alcanzaran cierto nivel crítico de efecto preclínico.

El "Perfil de Síntomas", el "Perfil de Signos" y el resultante "Indice del Perfil (véase la pág. 514)" son recursos para expresar convenientemente los resultados y merecen seria consideración. Sin embargo, esos procedimientos no comprueban los datos. Para que sean de utilidad han de estar basados en un cuidadoso examen del paciente. El índice del Perfil se debe usar o modificar en la forma que aconsejen la conveniencia y la experiencia.

Aunque no necesariamente relacionado con el uso del Indice del Perfil, se sugiere que se podría adiestrar a técnicos para que efectuaran estudios de los sistemas e incluso exámenes neurológicos sencillos. Los datos obtenidos por los técnicos podrían ser interpretados por un médico que examinaría detenidamente a los rociadores que presentaran anormalidades progresivas o suficientemente graves.

Aproximadamente la tercera parte de los rociadores que no estaban enfermos, mostraron alguna anormalidad en sus electroencefalogramas. Es interesante observar que la anormalidad de las ondas cerebrales puede continuar durante más de cuatro semanas después de la última exposición al dieldrín. Sin embargo, por las razones antes mencionadas, la prueba tiene un valor limitado.

No se ha determinado todavía la utilidad del examen del líquido cefalorraquídeo para el diagnóstico de la intoxicación por dieldrín, pero los hallazgos obtenidos justifican la realización de nuevos estudios en animales de laboratorio.

A pesar de los muchos estudios realizados, no se ha demostrado que tengan valor alguno las siguientes pruebas en relación con la intoxicación por dieldrín: el análisis de la orina, análisis completo de la sangre, radiografía torácica, la determinación de los campos visuales, las pruebas psicométricas y la determinación química de dieldrín en la sangre. El dieldrín se puede analizar satisfactoriamente después de agregarlo a sangre en el laboratorio (véase la pág. 527). Es posible que la dificultad de analizar químicamente el dieldrín en la sangre de los pacientes radique en la extracción y purificación de la muestra. Estos problemas son bien conocidos en relación con el análisis del DDT en sangre, en contraste con el análisis del DDT en grasa.

Muchos de los estudios antes citados terminan con una lista de recomendaciones sobre los métodos de rociamiento, recomendaciones que, en general, son razonables. Los autores afirman repetidas veces que el dieldrín se puede utilizar con seguridad en un programa de control de insectos si se siguen sus recomendaciones. Tal vez fuese así si éstas se aplicaran en la práctica. Sin embargo, hay que reconocer el hecho de que (salvo suspendiendo por completo el uso del dieldrín), no se ha logrado eliminar la intoxicación en ningún programa de control de insectos en que aquélla se produjo.

Hay que tener en cuenta las limitaciones de los conocimientos actuales sobre la intoxicación por dieldrín, y el fracaso de nuestros esfuerzos para eliminarla. Es igualmente importante recordar que (aun cuando no se dispone todavía de pruebas completas) existen indicaciones de que el reconocimiento médico ordinario (especialmente el estudio de los sistemas y el examen neurológico) representa el procedimiento más eficaz y práctico de descubrir la intoxicación preclínica. Si ésta se descubre temprano y se aleja permanentemente a los trabajadores afectados de la exposición al dieldrín, es posible que se eviten, en gran medida, los casos clínicos de intoxicación. Además, los fracasos anteriores no excluyen la posibilidad de que la protección a los rociadores y, especialmente, la adecuada enseñanza de la higiene personal a esos trabajadores, reduzcan considerablemente la incidencia de la intoxicación por dieldrín.

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Environmental Protection Agency

Behavioral and Physiological Effects of the Cholinesterase Inhibitor Carbaryl (1-Naphthyl Methylcarbamate)[1]

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SIDEROFF, S. I. AND J. A. SANTOLUCITO. *Behavioral and physiological effects of the cholinesterase inhibitor carbaryl* (1-*naphthyl methylcarbamate*). PHYSIOL. BEHAV. 9 (3) 459–462, 1972.—The anticholinesterase pesticide carbaryl was injected subcutaneously into rats lever-pressing for liquid reinforcement. During the test session a low intensity footshock accompanied each lever-press response with the carbaryl rats exhibiting a smaller response decrement due to the shock. In the second experiment, carbaryl injected rats displayed a higher behavioral threshold to footshock. These results appear to explain other studies in which animals given anticholinesterase agents show decreased efficiency in performing an avoidance response. Two additional experiments examined the effects of carbaryl on motivation for liquid. Rats lever pressing for liquid reinforcement on an FR-25 schedule were injected with carbaryl. It was found that the response onset after each reinforcement was significantly delayed in the carbaryl animals. This result, in addition to ad lib liquid consumption data, indicated a decreased drive for water in the carbaryl animals. Our findings, similar to results obtained using cholinolytics, presents an apparent discrepancy which we discuss in terms of (a) the necessity of having an optimum level of Acetylcholine (ACh) in the brain, (b) the possibility of a peripheral mechanism, or (c) the possibility of sympathomimetic effects of carbaryl.

Anticholinesterase   Avoidance conditioning   Carbaryl   Footshock   Pesticide   Pharmacology   Thirst

THE PESTICIDE carbaryl is a carbamate which produces cholinesterase inhibition [5]. Since its anticholinesterase activity occurs at doses lower than those causing signs of toxicity, discernible behavioral effects might also appear without symptoms of poisoning. A previous report by Giachetti, Chippi and Guerra [2] for example, found that low doses of another anticholinesterase, parathion, administered every other day for one month retarded the learning of a conditioned avoidance response. Similarly, introduction of an experimental carbamate, at doses well below toxic, levels considerably reduced the efficiency of rats in avoiding footshock [3].

We report here a series of experiments in which the effects of carbaryl administration on appetitive and aversive motivational properties in the rat are investigated. One objective of this study was to determine if the pesticide induced impairment of conditioned avoidance responses mentioned above was due to a learning or memory deficit or simply an increased tolerance to footshock. In addition we were interested in seeing if carbaryl instigated an increased rate of responding for liquid reinforcement, as would be expected from a cholinesterase inhibitor [4, 9].

In the first experiment, the rate of lever-press responding for liquid reinforcement was examined. In order to detect subtle differences in liquid motivation, a low-level footshock was introduced along with each lever-press. In the second experiment, thresholds to the footshock were examined in the control and carbaryl rats. In the third experiment we again looked for subtle differences in motivation for liquid by measuring the response onset latency after consumption was examined over a six week period with carbaryl injections given once a week.

EXPERIMENT 1

Method

Ten male Sprague–Dawley rats weighing between 200 and 250 g and approximately 60 days old, were used in Experiment 1. They were individually housed and placed on an ad lib food schedule. Each animal was water deprived for two days prior to bar-press training for water reinforcement.

Testing took place in a Lehigh Valley environmental cubicle and was controlled by electromechanical circuitry. Within the cubicle was a standard lever which, when depressed, activated a holding relay which permitted the lowering and raising of a water dipper with a capacity of 0.01 cc. The floor of the compartment consisted of a series of shock bars connected to a Lehigh Valley constant current stimulator and scrambler. Lever pressing was recorded by means of a Gerbrands cumulative recorder.

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[1] We thank Dr. N. Schneiderman for his helpful comments.

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The rats were trained to lever press for water on a continuous reinforcement schedule (CRF). Each session was 20 min long and was preceded by two days of water deprivation. Rats were allowed 30 min of free access to water prior to each deprivation period. After the rats were lever-pressing at a steady rate they were divided into experimental and control groups of 5 rats each. They were divided such that the groups' means were equal.

The 5 experimental animals were injected subcutaneously with a 1% solution of carbaryl in corn oil at 10 mg/kg (approximately 1/60 of the LD50). The 5 control rats were injected with corn oil. Preliminary investigations revealed that rats receiving this dosage of carbaryl exhibited a transient decrease in locomotor activity of 1–2 hr duration. Accordingly testing was always initiated two hours following injection. A 5 min baseline period of lever pressing for water was followed by a 20 min session during which each subsequent lever-press was accompanied by a 0.2 mA scrambled grid shock. Two test sessions were given, one week apart.

Results

The cumulative records of typical control and carbaryl injected subjects are shown in Fig. 1. During the baseline period the two animals display almost identical response rates. After onset of the contingent shock however, the control animal greatly reduced its rate of responding while the carbaryl animal showed a much smaller response decrement. The mean response rates of the 10 min shock period were 19.9% and 79.8% of the baseline for control and treated animals respectively. The difference was statistically significant ($t = 4.57$, $df = 18$, $p < 0.01$).

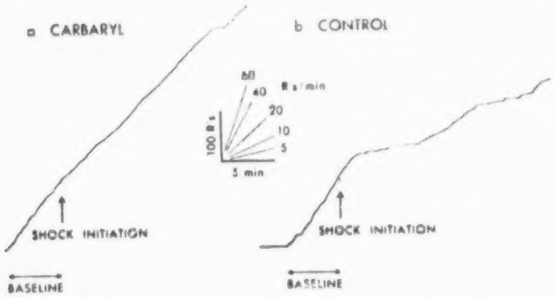

FIG. 1. Cummulative record of typical rats bar-pressing for water reinforcement on a CRF schedule. After 5 min baseline (at arrow), 0.2 mA footshock with each bar-press commenced.

EXPERIMENT 2

In this experiment, we were interested in whether the carbaryl group's performance of the previous test was due to a greater motivation for water, or to an increased tolerance to footshock.

Method

Ten naive male Sprague–Dawley rats, weighing 200–250 g were housed one to a cage and given food and water ad lib. The experimental enclosure consisted of metal walls and a clear Plexiglas top for observational purposes, and was 8.5 × 22.0 × 21.5 cm high. The floor consisted of shock-bars connected to a Nuclear-Chicago constant current stimulator.

Five experimental subjects were given 10 mg/kg of carbaryl subcutaneously in a 1% corn oil solution. Five control animals received just the corn oil. Two hr following injection each animal was placed in the enclosure and presented with a sequence of footshocks, first ascending and then descending in intensity. Shock was delivered only when the animal was motionless. Two observers, without knowing the treatment of the animals, independently assessed threshold intensity (as indicated by an "anticipatory" reaction or head turning response) and the intensity producing an aversive reaction (startle reaction or complete body movement). One week later the same procedure was repeated, i.e., the 5 treated and control animals were again injected with a carbaryl solution and oil respectively, and reactions of footshock were examined.

Results

The agreement between experimenters for scoring a stimulus-elicited "anticipatory" reaction and startle reaction, was 0.92 and 0.88 respectively. For both of these categories, carbaryl increased the response threshold. The mean stimulus intensities at which control and treated animals exhibited "anticipatory" reactions over the two testing sessions were 0.18 and 0.25 mA respectively. This difference was significant ($t = 2.84$, $df = 18$, $p < 0.02$). The stimulus intensity necessary to produce a startle reaction for control and treated animals were 0.34 and 0.48 respectively. This difference was also significant ($t = 2.45$, $df = 18$, $p < 0.05$). These results indicate an increased tolerance to shock in rats treated with carbaryl.

EXPERIMENT 3

The above experiment indicated an alteration in the effects of footshock on the carbaryl animals. While this was a probable cause of the results in Experiment 1, it was still possible that carbaryl was also affecting motivation for water.

Method

Sixteen male Sprague–Dawley rats weighing between 200–250 g and approximately 60 days old, were used in this experiment. The rats had free access to food and were placed on the water deprivation schedule used in Experiment 1. The apparatus used were also the same as in Experiment 1. The rats were trained to lever-press for water reinforcement on a CRF schedule. Once the animals were responding consistently, they were gradually raised to an FR-25 schedule. After a consistent lever-press rate was reached, each rat's mean time duration between each reinforcement and the subsequent onset of responding was calculated. The animals were matched on the basis of this response latency and divided into two groups. One group of 8 rats received subcutaneous injections of 10 mg/kg carbaryl in a 1% corn oil suspension. Eight control animals were given equivalent amounts of corn oil. In the subsequent test situation 2 hr after injection the average response latency was calculated over 80 reinforcements for each subject. The carbaryl subjects were then tested for 4 additional weeks. During this period, each rat received one injection of 10 mg/kg/week. They were tested 2 hr after each injection and again 48 hr later.

Results

The responding of 2 carbaryl rats broke down on the first injection day within the first 10 reinforcements. On this test day the average latency for the remaining 6 animals was 6.43 (±S.E. 0.86) sec as opposed to 3.75 (±S.E. 0.29) sec for the control group. This difference between treated and control group latencies was significant ($t = 3.31$, $df = 12$, $p < 0.01$). Examination of responding for the 6 carbaryl animals over the 4 additional treatment weeks revealed no significant change from the latencies on the first carbaryl day ($p < 0.02$).

EXPERIMENT 4

In this experiment we attempted to more clearly demonstrate the results of the previous experiment showing a decreased motivation for liquid by the carbaryl animals.

Method

Eighteen male Sprague-Dawley rats weighing between 200–250 g were housed one to a cage and allowed food and water ad lib. Body weight and water consumption were measured over a one-week period, at which time the rats were divided into 2 groups of 9 animals matched with respect to weight and water consumption. The carbaryl and the control groups were then injected once a week, for 5 weeks, as described in the previous experiments. The amount of water consumed by each rat was measured twice a week and its body weight taken just prior to dosing. Water bottles were fitted with ball-valve drinking tubes to minimize wastage. Water consumption was determined by weigh-back and expressed in grams.

Results

Figure 2 shows liquid consumption for the control and experimental groups over the baseline and treated weeks. It can be seen that the carbaryl animals showed a marked reduction in liquid intake. The mean water consumption of the first plus the last week and a half was obtained for each group. Statistical analysis comparing these means showed a significant difference ($t = 3.40$, $df = 106$, $p < 0.01$). The mean body weights of the two groups did not differ significantly over the five-week period ($p < 0.2$).

DISCUSSION

The results of Experiment 1, at first, indicated that rats injected with carbaryl when water deprived, have a greater drive for water than control animals under similar deprivation. In the first part of this experiment, in view of the results showing that both groups lever-pressed at similar rates, it was assumed that we were observing the upper limit of responding. In order to bring the baseline rate below this limit to observe any effects of carbaryl, the instantaneous, low level footshock was delivered with each lever-press. This, in effect, made the task more difficult and decreased the rate of responding for both groups; however, the control groups rate was significantly lower than the carbaryl groups. While the initial interpretation indicated an increased motivation for liquid produced by carbaryl, Experiment 2 yielded another possibility. In this experiment we found that both the "anticipatory" and the "startle" response thresholds in the rats were significantly higher in the carbaryl group. This increased tolerance to shock in subjects treated with carbaryl may account in part for the difference in the shock induced decrements between groups.

While the evidence indicated the importance of the difference in sensitivity to shock, it was still conceivable that carbaryl as a cholinesterase inhibitor enhanced the animals' drive for water [9, 14]. In the third experiment, however, it was found that carbaryl injected animals consistently exhibited longer latencies in returning to the bar after receiving water reinforcement. Also, in the last experiment, the treated animals exhibited a decreased daily water intake. These results indicate that carbaryl reduces water consumption, thus suggesting that this pesticide influenced motivation in both appetitive and aversive situations.

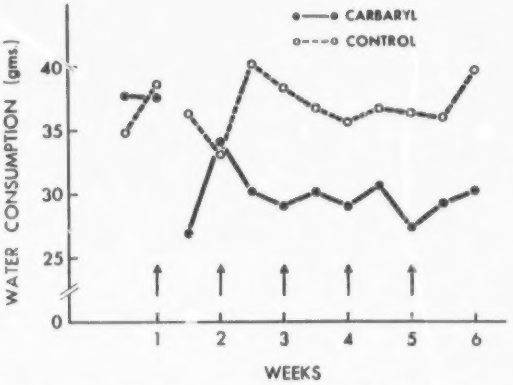

FIG. 2. Average daily water consumption in carbaryl and control groups, measured twice a week. The dosing is indicated by arrows.

The increased tolerance to shock induced by carbaryl in the present study affords a possible explanation for deficits in avoidance conditioning reported by Giachetti *et al.* [2] and Goldberg *et al* [[illegible]]. Conceivably, in these experiments, there was less motivation to avoid or escape since the animals were less sentive to the footshock reinforcer. The result was less efficient performance. This would appear to be a more parsimonious explanation than any deficit in memory or learning.

These results are interesting in light of recent work indicating the same findings from introduction of cholinolytics [6, 10, 12]. These apparently contradictory results can be accounted for by an hypothesis put forth by Deutsch [1], whereby cholinergic pathways require an optimal concentration of acetylcholine to function properly. Thus, it is possible that too much or too little endogenous acetylcholine in central synaptic pathways could produce similar effects on the same system.

Interestingly, Khavari and Russell have published evidence that cholinolytic drugs including atropine and methyl atropine reduce water intake [7]. Since the latter drug does not pass through the blood brain barrier very effectively the authors indicate a probable peripheral action. Furthermore, they find decrements in drinking at dose levels which do not produce significant changes in central ACh levels. Thus, there might be a peripehral mechanism analagous to the central theory discussed above, in which an optimal peripheral level of ACh is required for normal functioning.

An alternative explanation is suggested by other observations in our laboratory which show that carbaryl has sympathomimetic properties on smooth muscle preparations *in vitro* [11]. If similar properties were found in the central nervous system it might support the contention of Leibowitz [8], that alpha adrenergic agonists activity suppress water consumption.

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TOXICOLOGY PROGRAM
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DDA Excretion Levels

Studies in Persons With Different Degrees of Exposure to DDT

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THE POTENTIAL health hazard of pesticides in general and of the persistent chlorinated hydrocarbon insecticides in particular has been the subject recently of much public interest.[10] An important aspect of study of the health hazard of these materials is an evaluation of the magnitude of exposure sustained by the worker in his occupational contact during manufacture, formulation, or application; by the resident who receives drift of spray or dust into his home when the pesticide is used nearby; and by the individual in the general population who consumes residues of these pesticides in his daily diet.

The magnitude of exposure to DDT has been determined by measurement of DDT and DDE storage levels in body fat[8] or, less commonly, by estimation of urinary excretion levels of the relatively water-soluble metabolite DDA (dichlorodiphenyl acetic acid).[2] It is, of course, much easier to obtain urine samples for DDA analysis than to procure surgically the fatty tissue required to measure DDT and DDE storage levels.

Storage of DDT and DDE has been determined in the general population, in meat abstainers, in various environmental and occupational groups,[7] and in volunteers given known oral doses of DDT.[5,6] DDA excretion has been measured previously in workers in manufacturing and formulating plants[9] and in volunteers given known oral doses of DDT.[5,6]

The present study involved measurement of the DDA excretion levels in persons in the general population who ate an average diet and had no environmental or occupational exposure to DDT. Excretory levels of a small number of meat abstainers and of persons who had been environmentally or occupationally exposed to DDT are also included.

The work described in this paper is a component part of a more comprehensive study. The complete project involves determination of DDT and DDE storage levels in body fat in the general population and in specific environmental and occupational groups, measurement of DDT and DDE content of complete prepared meals, and determination of DDT and DDE excretion in milk by nursing mothers. These surveys have been correlated by obtaining as many of the samples as possible from the same individuals. For example, a number of the subjects in the present study also contributed fat for determination of DDT and DDE storage levels. The results of the other segments of the complete DDT study will be published separately.

Materials and Methods

A total of 107 urine samples were analyzed for DDA content using the procedure of Cueto et al.[2]

Each subject who contributed a urine sample was classified according to history into one of several exposure groups numbered in approximate order of increasing exposure. The identity of these exposure groups was essentially the same as that used in the previous survey of DDT and DDE storage levels.[7] The identity of these groups from whom samples were obtained for this study is as follows:

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TABLE 1.—*Excretion of DDA in Urine of People in the United States with Regard to Dietary, Environmental, or Occupational Exposure to DDT*

Exposure Group No.	Exposure Group Description	No. of Cases	Range of DDA Concentration in Urine (ppm)
2	Abstainers from meat	4	<0.02
3	General population	79	<0.02-0.35
4	People with heavy environmental exposure	13	<0.02-0.11
5&6	Agricultural applicators	11	<0.02-0.17

Group 2: Abstainers from meat (4 cases).

Group 3: General population (persons eating meat more than 12 times a month and without heavy environmental or occupational exposure) (79 cases).

Group 4: Heavily environmentally exposed residents living within 500 feet (152) meters of agricultural application (13 cases).

Group 5: Former applicators (more than a year ago) (8 cases).

Group 6: Applicators (within the last year). For inclusion in this classification a person had to have applied DDT in some agricultural or other commercial fashion at least twice during the past year (3 cases).

No samples in Group 1 (persons who died before the advent of DDT in the United States) were available for analysis in the present study. The numbers of subjects in Groups 5 and 6 were small and the results for them are considered together.

Twenty-three of the individuals in the general population group in this paper also contributed fat for the determination of DDT and DDE storage levels in the general population in a related study.[11]

Results and Comment

The excretion of DDA in urine by people in various dietary, environmental, and occupational exposure groups with regard to DDT contact is shown in Table 1.

The DDA excretion levels for the general population group ranged from below the experimental limit of the analytical method, which was about 0.02 ppm, to 0.35 ppm. Of the 79 samples in this group which were tested, 56 samples (74%) did not contain detectable quantities of DDA. The results shown in the table did not indicate any important difference in DDA excretion level between the general population and any other group studied. It is entirely possible that the DDA excretion level in meat abstainers is lower than that for the general population; however, it is impossible to reach a firm conclusion on the basis of the small number of samples obtained from meat abstainers.

Twenty-three persons from the general population group also contributed fat in the study to

TABLE 2.—*Excretion of DDA and Storage of DDT by People of the United States as Determined by Degree of Dietary, Environmental, Occupational, or Investigational Exposure*

Type of Exposure and Name of Group	DDA Excretion			DDT Storage			Reference
	No. of subjects	Range, ppm	Mean ppm±SE	No. of subjects	Range, ppm	Mean, ppm±SE	
Average exposure							
Volunteers (controls)	8	<0.05	—	8	2.9-9.3	6.9±0.76	6
Same	8	<0.02-0.07	—	10	2.6-5.8	3.7±0.37	5
General population	23	<0.02-0.18	—	23	0.7-8.9	3.0±0.4	This paper
Heavy environmental exposure							
Residents near treated fields	—	—	—	110	2-14	6.0±0.3	7
Same	13	<0.02-0.11	—	—	—	—	This paper
Same	—	—	—	28	0.4-16.8	4.3±0.7	11
Light occupational or investigational exposure							
Agricultural applicators	11	<0.02-0.17	—	—	—	—	This paper
Same	—	—	—	30	3-37	14.0±1.5	7
Volunteers (3.5 mg/man/day)	2	0.10-0.42 *	0.21±0.06 *	2	26-33 †	29.5±3.5 †	6
Same	6	0.06-1.98 ‡	0.23±0.04 ‡	6	39-76 §	50.2±5.0 §	5
Heavy occupational or investigational exposure							
Formulating plant workers	40	0.12-7.56	1.71±0.23	—	—	—	9
Same	—	—	—	2	122-648	—	6,8
Volunteers (35 mg/man/day)	6	0.69-9.67 *	2.[illegible]±0.53 *	6	68-367 †	231±42.9 †	6
Same	6	0.18-9.21 ‡	3.09±0.02‡	6	105-619 §	281±79.5 §	5

* Based on all samples after 35th week of dosage.

† Based on the latest samples from each man (11 months or more after dosage started in each instance).

‡ Based on all samples from the 35th through the 93rd week after dosage started.

§ Based on samples taken 21.5 months after dosage started.

determine the storage level of DDT and DDE. The DDA excretion levels for this subgroup ranged from <0.02 to 0.18 ppm. Sixteen samples (70%) from this subgroup did not contain detectable quantities of DDA. Thus, the distribution of DDA excretion levels for the subgroup contributing fat was about the same as that for the entire group studied. The 23 individuals in this subgroup had mean storage levels of 3.0 ppm for DDT and 7.3 ppm for DDE. These values may be compared with levels of 4.0 ppm for DDT and 7.8 ppm for DDE which were determined for the entire general population group of 130 persons.[11] There was no important difference between the DDT or DDE levels for the subgroup and for the entire group, thus indicating that the smaller population sampled for DDA excretion was representative of the entire group which was studied for DDT and DDE storage in fat.

The results of the present study of DDA excretion for the general population and for various environmental and occupational groups are shown in Table 2 in relation to other published studies in which this parameter has been measured. DDT storage levels for these groups are also shown when available.

The absence of any detectable difference in DDA excretion levels among groups with light to moderate exposure has been noted above. However, the DDT storage values are distinctly different for two of these groups—the general population and agricultural applicators—as can be seen in the Table. The reason for the difference between these groups in the case of storage and for their similarity in the case of excretion is not clear. It may well be that further and more exhaustive study of the pharmacodynamics of the equilibrium between DDT stored in fat and DDA excreted in urine will provide the explanation. There seems to be some indication that the DDA excretion level can approach the general population value, while the DDT storage level in fat is still significantly elevated.

Thus, the present method of measurement of DDA excretion does not seem to be precise enough to indicate differences in this parameter correlated with observed intergroup differences in DDT storage level within the relatively narrow dosage range associated with light to moderate exposure. There is a general correlation between DDA excretion and DDT storage for groups with widely different degrees of exposure. However, the rate of change of these two parameters with increase in DDT exposure does not seem to be the same. DDA excretion apparently increases disproportionately at the higher exposure levels.

In view of the above-noted lack of any detectable difference in DDA excretion level among groups with light to moderate exposure, no importance can be attached to the fact that there was reasonably good correspondence among the three studies (Table 2) of DDA excretion in the general population. The same conclusion applies to the failure to detect an effect of environmental exposure to DDT (Group 4) on the excretion of DDA. It will be recalled that even the difference in storage between Group 4 and the general population (Group 3) is small[7]; in fact, the most recent study failed to demonstrate any difference.[11]

Based on an average daily urine output of 1.5 liter and a DDA excretion level range of from <0.02 to 0.35 ppm, calculations indicate that the daily DDA excretion for the general population was between <0.03 and 0.53 mg. Thus, about 75% of the general population excrete less than 0.030 mg of DDA per day or the equivalent of less than 0.038 mg of DDT. It has been found by the analysis of typical meals that the average person in this country consumes about 0.038 mg of DDT per day in his food.[8] Thus, the results of these two studies are not inconsistent. However, it is apparent that present data on the urinary excretion levels of DDA in the general population are insufficient to support any statement concerning the fraction of the daily DDT intake which is accounted for as urinary DDA.

There is a need for a more sensitive method for determination of DDA excretion in urine. This need is particularly great with respect to the general population who excrete quite low levels of this metabolite in association with their low-level exposure to DDT. It is hoped that gas chromatographic methods will provide the solution to the problem of greater sensitivity. However, the acidic nature of the DDA molecule seems to interfere with its passage through the chromatographic column. It has been suggested that esterification of the DDA molecule could be carried out to cut down reactivity and thus aid passage through the column.

DDA is much less toxic than DDT and would appear to contribute little to the acute toxic hazard of the parent compound. The acute oral LD_{50} values to male white rats are 740 mg/kg for DDA and 113 mg/kg for DDT.[4] However, the long-term effects of DDA have not been well studied.

Bleiberg and his colleagues have recently shown that in the rabbit DDA inhibits choline acetylation, apparently by blocking acetate activation, and decreases intestinal motility.[1] These studies were carried out in in vitro systems or on isolated organs. Similar effects have not yet been reported in whole animals.

Summary

The excretion of DDA in urine by people in various dietary, environmental, and occupational exposure groups with regard to DDT contact was determined.

The DDA excretion levels for the general population ranged from below the experimental limit of the analytical method (0.02 ppm) to 0.35 ppm. Of the 79 samples in this group which were tested, 56 (74%) did not contain detectable quantities of DDA.

There was no indication that any important difference in DDA excretion level existed among the general population, meat abstainers, or people with heavy environmental or light occupational exposure. However, striking differences in DDA excretion exist for groups with widely different degrees of exposure.

A more sensitive method for determination of DDA excretion levels in urine is needed, since the present procedure gives values below the limit of sensitivity for about three fourths of subjects from the general population group.

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Organophosphorus Insecticides and Mental Alertness

Studies in Exposed Workers and in Poisoning Cases

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All agricultural workers are subjected to some risk of poisoning by the insecticides they use. For agricultural workers who use complicated, moving machinery, in addition to the risk of overt poisoning by pesticides, one must consider the possibility that exposure to these chemicals might adversely affect mental alertness, a consideration of particular importance in the case of spray pilots who must be constantly alert.

It is well known that there is a distinct central nervous system component in the symptom complex of severe clinical poisoning by the organic phosphorus compounds.[6] In fact, the signs and symptoms of poisoning have been divided classically into nicotinic, muscarinic, and central effects. The central nervous system manifestations of organic phosphorus poisoning include tension, anxiety, nervousness, restlessness, and giddiness. Sleep difficulties with excessive dreaming and nightmares, difficulty in concentrating, impairment of memory, and slowing of reactions may occur. In occasional persons apathy, withdrawal, and depression occur.[7] Changes in electroencephalographic tracings correlated with mental effects have been observed in persons poisoned by the organic phosphorus compounds developed for chemical warfare use as "nerve gases." Mental effects generally have been more notable with nerve gases than with insecticides.

It is important to know whether or not mental effects may precede or take place in the complete absence of other, more disabling signs and symptoms of intoxication. The primary purpose of this study reported here was to attempt to answer this question.

From case reports, it seemed that "mental confusion," the predominant symptom in a few instances, consisted primarily of lapses of attention. The tests herein described were designed to test the hypothesis that (*a*) lapses of attention—whether due to "blanking out," "inappropriate sense of well-being," or other causes—are relatively common in persons with extensive exposure to organic phosphorus insecticides, but (*b*) these lapses come to attention only when they are sufficiently prolonged and occur during critical situations, such as the operation of aircraft. The alertness tests used in this study were selected primarily on the basis of their capability to measure lapses of attention. Also, they give some measure of other altered mental functions, such as decreased judgment, although their sensitivity for this purpose is less than for determining lapses of attention.

Materials and Methods

Tests of mental alertness were carried out during 1960, 1961, and 1962 on groups of people with varying degrees of exposure to organic phosphorus pesticides and on persons with no known exposure

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From the Toxicology Section, Technology Branch Communicable Disease Center, Public Health Service, US Department of Health, Education, and Welfare.

to these materials as controls. Tests were administered before the spray season, at intervals during the season (with an effort being made to test each individual immediately after his periods of greatest exposure), and after the season. The control groups were tested at about the same time as the corresponding insecticide worker groups. Test results for the control group were tabulated into exposure and nonexposure periods based on the status of the corresponding insecticide worker group at the time of testing.

Each time a subject was tested he was questioned in detail concerning recent exposure to pesticides. Data were also recorded on recent or current illnesses, intake of drugs or alcohol, sleep schedules (particularly for the previous night), fatigue, and each person's estimate of his present physical state.

1960 Survey.—During 1960, the exposure group consisted of 53 persons, including 17 mixing-plant personnel, 16 ground applicators, 14 pilots, four loaders and flagmen, and two warehousemen. Twenty-five persons with no known occupational or environmental exposure were studied as controls. A total of 235 different tests were made.

During 1960, two different paper and pencil tests furnished by Dr. Ardie Lubin of the Walter Reed Institute of Medical Research were used. All tests were carried out for a standardized, ten-minute period. One was the Gersoni U test, a self-paced vigilance test of the cross-out type consisting of three sheets of capital letters in randomized order, arranged 30 letters per line, and 50 lines per page. There were two forms of this test, designated A and B, which differed only in regard to the order of the letters on the page. Each subject received forms A and B alternately during the course of the year's testing program. The subject was instructed to draw a slanting or vertical line through every "R."

The tests were scored for errors of omission, commission, and for lines completed. Templates prepared from exposed film were used in scoring.

The other procedure was the EX test, another self-paced vigilance test, using a question sheet and an IBM answer sheet for true-false answers. These question sheets contained a list of 600 letter-groups (not necessarily words) of ten letters each. The subject was instructed to inspect each letter-group for the presence of the letters "E" and "X." If either letter alone was present, the correct answer was "true." If neither letter was present in the letter-group or if both letters were present, the correct answer was "false." Scoring was by machine; the number of answers correct, incorrect, and total attempted were noted.

Most subjects tested during 1960 were given both the Gersoni U test and EX test.

1961-1962 Survey.—During 1961 and 1962 the exposure group consisted of 68 persons, including 27 mixing-plant personnel, 25 ground applicators, nine pilots, and seven loaders and flagmen. Twenty-two controls were studied. A total of 275 tests were conducted, of which 189 fell into a series that permitted useful study.

In both 1961 and 1962, mental alertness was evaluated by determining complex reaction time using the machine described by Street.[illegible] Briefly, the stimuli were five lights of different colors that flashed in random order at varying intervals so that little or no learning was possible. The subject was required to press one of two levers corresponding to each of two lights and to make no response to the other lights. The time interval between stimulus and response was measured. Errors of omission (missed responses) and errors of commission (improper responses) were recorded. The test period was ten minutes. In 1961, the machine was used with a kymograph; the parameters used to grade performance were determined by study of the tracings. Grades were based only on the final five-minute portion of the ten-minute test period. In 1962, a more sophisticated version of the machine was used in which responses occurring during various time intervals—less than 0.2 second, 0.2 to 0.4, 0.4 to 0.6, 0.6 to 0.8, and greater than 0.8 second—were recorded and summed electronically, as were errors of omission and errors of commission.

Clinical Studies.—In addition to the planned surveys of 1960, 1961, and 1962, clinical study was also made of a number of cases of organic phosphorus poisoning that occurred in the Pacific Northwest between 1960 and 1963. While there was no mandatory system for reporting cases to our laboratory, cooperation of physicians treating cases was excellent. Reporting was encouraged by providing physicians with advice on diagnosis and treatment, necessary laboratory services (blood cholinesterase and urine *p*-nitrophenol determinations) in study cases, and a supply of the new, experimental antidote pralidoxime chloride when need was established by telephone consultation.

A total of 187 cases of suspected organic phosphorus poisoning was studied during the four-year period. After preliminary judgment as to whether or not intoxication with pesticides was involved, each organic phosphorus poisoning case was evaluated on the basis of relative severity of somatic illness and mental effect to observe any effect organic phosphorus exposure might have on mental alertness when no physical signs or symptoms were detectable.

A total of 33 tests of mental alertness were carried out on 11 selected cases of suspected poisoning. The tests were given only to persons with mild illness or to more severely affected individuals during the recovery period.

Results

1960 Survey.—The results of the Gersoni U test and the EX test for the various exposure groups and the controls are summarized in Tables 1 and 2. The insecticide worker groups are arranged in approximate order of decreasing estimated exposure to organic phosphorus materials. Although the tests were given at about one-month intervals, the scores are not shown in the Table for each test interval but are combined under total exposure or nonexposure period. However, in the text below some test results are cited for each successive test interval for some groups, particularly in the discussion of learning. It should be noted that any effect of exposure to organic phosphorus insecticides on performance should be detectable in each of three different comparisons: (1) decreasing effect correlated with decrease in exposure for the five exposure groups, (2) differences between exposed and control groups, and (3) differences for exposed group (both as individual groups and *in toto*) between nonexposure and exposure periods.

With the Gersoni U test, although the subjects made many errors of omission (range, 0 to 165), they made very few errors of commission (range, 0 to 7). However, with the EX test there were a substantial number (range, 0 to 71) of incorrect answers.

On both tests normal unexposed individuals varied widely in levels of attainment. For a given individual, however, the scores attained on the same test at different times were fairly uniform. The test scores indicate that very little learning occurred. The lack of progressive improvement on successive tests for the control group with respect to errors of omission (successive mean scores: 15.3, 21.7, 16.3, 19.8, and 14.4) and errors of commission (0.1, 0.5, 0.1, 0.2, and 0.4) on the Gersoni U test and with respect to incorrect answers (9.9, 11.9, 10.0, 10.5, and 9.9) on the EX test shows that no significant degree of learning occurred for these parameters. There seemed to be some learning in other parameters measured, including lines completed (successive mean scores for control group: 63.1, 69.9, 70.2, 76.2, and 72.3) on the Gersoni U test and correct answers (135, 141, 153, 151, and 169) and number of questions answered (145, 153, 164, 162, and 179) on the EX test. Much of the learning which apparently took place in these instances was evident on the second test.

Generally, younger subjects made better scores on both tests than did older persons. The pilot group was outstanding for the small number of errors made on tests during periods of nonexposure. On the average, the pilots made fewer errors of omission

TABLE 1.—*Performance on Gersoni U Test for Various Occupational Groups Exposed to Organic Phosphorus Pesticides and for a Control Group*

Exp Group	No. of Persons	No. of Tests		Value	Errors of Omission		Errors of Commission		Lines Completed	
		Nonexp	Exp		Nonexp	Exp	Nonexp	Exp	Nonexp	Exp
Mixing plant personnel	17	9	48	Range	3-21	1-121	0-2	0-3	49.0-78.9	55.2-79.8
				Mean ±SE*	10.3±2.5	22.1±4.3	0.22±0.22	0.17±0.08	62.4±2.2	59.8±1.6
Ground applicators	16	11	36	Range	2-165	1-54	0-4	0-2	61.0-93.0	47.1-84.3
				Mean ±SE	17.0±6.1	17.0±4.4	0.63±0.30	0.21±0.09	77.0±3.2	69.1±1.5
Pilots	14	14	28	Range	0-23	1-40	0-1	0-1	44.0-75.5	48.0-74.0
				Mean ±SE	10.1±1.6	14.1±2.2	0.07±0.07	0.07±0.05	61.1±2.2	60.1±1.4
Loaders and flagmen	4	4	3	Range	6-16	4-21	0-1	0	46.3-55.1	42.4-62.0
				Mean ±SE	11.0±2.9	12.7±4.9	0.25±0.25	0	50.7±1.9	53.6±5.4
Warehousemen	2	0	4	Range	—	29-43	—	0-1	—	55.0-67.0
				Mean ±SE	—	35.0±3.5	—	0.25±0.25	—	60.0±2.6
Total exposed group	53	38	119	Range	0-165	1-121	0-4	0-3	44.0-93.0	55.2-92.0
				Mean ±SE	12.2±1.0	18.8±2.3	0.29±0.13	0.16±0.04	64.8±1.0	62.7±0.9
Control	25	37	41	Range	0-61	0-86	0-4	0-7	43.7-94.5	48.0-95.0
				Mean ±SE	15.0±3.1	20.0±3.3	0.19±0.12	0.41±0.10	66.8±2.1	70.9±1.9

* Standard error of the mean.

TABLE 2.—*Performance on EX Test for Various Occupational Groups Exposed to Organic Phosphorus Pesticides and for a Control Group*

Exp Group	No. of Persons	No. of Tests		Value	Correct Answers		Incorrect Answers		No. of Questions Answered	
		Nonexp	Exp		Nonexp	Exp	Nonexp	Exp	Nonexp	Exp
Mixing plant personnel	17	9	44	Range	131-185	83-186	0-13	1-41	138-190	97-180
				Mean ±SE *	151±6.1	136±3.3	5.2±1.1	10.8±1.2	158±6.3	147±3.3
Ground applicators	16	11	36	Range	80-234	26-206	2-71	2-36	135-238	87-214
				Mean ±SE	161±13.1	147±5.9	16.8±5.9	11.2±1.4	168±9.0	159±5.5
Pilots	14	14	28	Range	97-200	78-191	1-17	0-35	106-204	91-192
				Mean ±SE	137±8.7	127±5.7	6.4±1.4	9.9±1.8	143±8.8	137±5.1
Loaders and flagmen	4	4	3	Range	76-170	156-171	5-10	4-24	86-177	168-180
				Mean ±SE	127±21.0	161±4.8	7.5±1.0	11.2±6.4	134±20.6	173±5.0
Warehousemen	2	0	4	Range	—	66-130	—	6-30	—	85-150
				Mean ±SE	—	97±13.9	—	11.7±2.9	—	109±14.5
Total exposed group	53	38	117	Range	76-234	26-206	0-71	1-41	86-238	87-214
				Mean ±SE	142±5.3	137±2.9	9.2±1.9	10.8±0.9	152±5.0	149±2.7
Control	25	22	41	Range	78-236	68-223	1-45	0-45	97-260	108-292
				Mean ±SE	151±6.9	146±4.7	9.9±1.8	11.1±1.6	161±6.3	157±4.1

* Standard error of the mean.

(pilots, 10.1±1.6; controls, 15.0±3.1) and of commission (pilots, 0.07±0.07; controls, 0.19±0.12) on the Gersoni U test and gave fewer incorrect answers (pilots, 6.4±1.4; controls, 9.9±1.8) on the EX test. Only one error of commission on the Gersoni U test was committed by the entire pilot group on preexposure and postexposure tests combined. The pilots were somewhat slower and more deliberate in their work as shown by the fact that, on the average, they completed fewer lines (61.1±2.3) on the Gersoni U test than did the control group (66.8±2.1), and answered fewer questions (143±8.8) on the EX test than did the control group (161±6.3). The differences between the pilots and the control group cannot be accounted for on the basis of age.

On no parameter of either test was there any indication of variation in effect between the exposure groups correlated with differences in amount of exposure.

The differences in attainment with respect to errors of commission on the Gersoni U test and to all three parameters measured on the EX test between the entire exposure group and the control group were quite small and of doubtful significance. As to errors of omission on the Gersoni U test and with respect to all three parameters measured on

TABLE 3.—*Performance on Complex Reaction Time Test for Various Occupational*

Exp Group	No. of Persons	No. of Tests		Value	Errors of Commission		Errors of Omission	
		Nonexp	Exp		Nonexp	Exp	Nonexp	Exp
Mixing plant personnel	27	27	43	Range	0-10	8-60	0-2	0-5
				Mean ±SE *	3.1±0.44	3.1±0.28	0.33±0.12	0.48±0.16
Ground applicators	23	15	12	Range	0-10	0-15	0-1	0
				Mean ±SE	3.5±0.67	5.2±1.20	0.13±0.09	0
Pilots	9	16	18	Range	0-9	0-10	0-1	0-1
				Mean ±SE	2.2±0.66	2.8±0.74	0.06±0.06	0.11±0.08
Loaders and flagmen	7	7	5	Range	0-9	1-3	0-1	0-4
				Mean ±SE	3.0±1.5	2.0±0.57	0.14±0.14	1.0±0.77
Total exposed group	66	65	79	Range	0-10	0-60	0-2	0-5
				Mean ±SE	3.0±0.31	3.3±0.35	0.20±0.06	0.25±0.10
Controls	22	26	30	Range	0-18	1-15	0-1	0-5
				Mean ±SE	3.8±0.65	5.9±1.5	0.16±0.06	0.32±0.24

* Standard error of the mean.

the EX test, the achievement of the exposed group as a whole was lower during the exposure period than during the nonexposure period. However, based on these measurements, the control group also did poorer during the "exposure" period than during the "nonexposure" period. This finding seems to indicate that the poorer performance during the exposure period may have been related to some factor other than contact with insecticides.

On the Gersoni U test, there was a decrease in errors of omission correlated with decreasing exposure for four of the five occupational groups studied as seen in Table 1. This progression did not extend to the group with the lowest expected exposure (warehousemen). No nonexposure tests are available for the two persons in this group. However, the mean errors of omission for the total exposed group, including the warehousemen, during the exposure period (18.8±2.3) is about the same as that for the control group (20.0±3.3).

With respect to lines completed on the Gersoni U test, the exposure group as a whole showed a poorer score during the exposure period (62.7±0.9) than during the nonexposure period (64.8±1.9). In contrast, the control group scored better on this parameter during the exposure period (70.9±1.9) than during the nonexposure period (66.8±2.1).

1961-62 Survey.—The results on the complex reaction time test for the various exposure groups and the controls are summarized in Table 3. The tabulated data include number of: errors of commission, errors of omission, responses occurring earlier than 0.38 second, responses occurring earlier than 0.50 second, responses occurring later than 0.50 second, and average reaction time.

In contrast to performance on the Gersoni U test, the subjects made numerous errors of commission (range, 0 to 69), but few errors of omission (range, 0 to 5). The highest recorded number of errors of commission (69) was registered by a single exposed subject on two different occasions. The greatest number of errors of omission (five) was noted for a mixing plant worker and a control subject, each on only one occasion.

There was a wide range of performance among normal individuals on this test. However, performance for a given individual at different times was relatively constant. There was little, if any, indication that an important amount of learning occurred as shown by a lack of progressive improvement in the mean performance of the control group on successive tests judged by number of errors of commission (5.1, 4.7, 5.6, 10.3, 2.3, 3.2, and 4.7) and omission (0.3, 0.9, 0.3, 0, 0.2, 0, and 0.2), number of responses occurring earlier than 0.38 second (9.4, 5.5, 8.0, 14.0, 5.7, 12.0, and 10.5), number of responses occurring earlier than 0.50 second (39.5,

Groups Exposed to Organic Phosphorus Pesticides and for a Control Group

Responses Earlier Than 0.38 Sec		Responses Earlier Than 0.50 Sec		Responses Later Than 0.50 Sec		Mean Reaction Time (Sec)	
Nonexp	Exp	Nonexp	Exp	Nonexp	Exp	Nonexp	Exp
0-33	0-21	1-81	9-69	0-69	1-51	0.37-0.69	0.44-0.64
8.2±1.7	4.6±0.80	38.6±4.3	35.1±2.9	15.8±2.7	18.2±2.0	0.52±0.01	0.53±0.01
0-23	0-6	3-70	10-87	1-64	0-22	0.40-0.68	0.49-0.69
9.1±2.0	4.0±0.75	42.8±7.2	39.0±4.3	19.6±3.1	21.6±4.6	0.52±0.03	0.55±0.03
0-39	0-39	26-89	25-85	0-50	0-32	0.37-0.58	0.35-0.61
10.3±2.0	12.4±2.8	62.9±5.4	64.5±2.8	6.5±2.0	8.9±2.3	0.48±0.02	0.46±0.02
6-31	5-19	24-77	31-86	0-23	11-21	0.36-0.40	0.47-0.56
22.1±4.2	11.3±2.9	63.4±7.4	44.[illegible]±4.9	6.4±2.9	14.7±2.4	0.41±0.02	0.52±0.09
0-39	0-39	1-89	8-86	0-64	0-51	0.37-0.69	0.38-0.66
12.0±1.3	6.7±0.90	48.3±3.1	40.0±2.2	13.3±1.8	16.3±1.5	0.49±0.01	0.52±0.01
0-38	0-24	0-84	2-79	0-62	3-64	0.33-0.69	0.40-0.81
9.5±1.6	7.8±1.6	44.8±4.3	40.5±5.3	15.0±3.2	18.3±3.6	0.50±0.02	0.52±0.02

35.3, 46.4, 40.3, 47.1, 48.2, and 49.8), number of responses occurring later than 0.50 second (14.3, 21.9, 16.5, 12.3, 14.3, 15.2, and 20.5), and mean reaction time (0.51, 0.55, 0.51, 0.49, 0.51, 0.50, and 0.50 seconds).

It is interesting to note in Table 3 that, with regard to every parameter studied, both combined exposed and control groups scored about as well or better during nonexposure than exposure periods. However, in every instance, with the possible exception of responses earlier than 0.38 second, the differences were small for both groups and as large or larger for the controls as for the exposed. Even for responses earlier than 0.38 second, though there was a larger variation in score between nonexposure and exposure periods for the entire exposed group (12.0±1.3 to 6.7±0.9) than for the controls (9.5±1.6 to 7.8±1.6), there was no significant difference between actual mean scores of the two groups for the exposure period. Thus, there was no indication from complex reaction time test results that exposure to organic phosphorus insecticides had any significant effect on mental alertness.

Three pilots in the survey crashed during the period of participation. One spray pilot in the 1960 studies was killed on July 7, 1960, in an airplane accident while on a cropdusting mission. His plane hit a power line. He was spraying a herbicide (2,4-D) at the time of the accident and for several days previously had had exposure to 2,4-D only. There was no indication that spray exposure was related in any way to his accident. He had been tested last by us on May 31, 1962, after some early season exposure. His performance at that time was: Gersoni U test: errors of omission, 17; errors of commission, 0; lines completed, 573. EX test: right, 128; wrong, two; questions answered, 130. These test results are well within the range of variation for the general population but are near the lower range of scores for pilots during periods of nonexposure. No preexposure test was done on this man.

One of the other airplane accidents involved power failure on a helicopter in flight; the other involved running out of gasoline by a winged aircraft in flight. Both machines in these accidents were heavily damaged, but the pilots were not injured. The power failure incident involved malfunction of equipment rather than deficiency on the pilot's part. Lack of judgment on the part of the pilot for allowing his gasoline supply to become depleted could have been involved in the other incident. This man was consistently one of the poorest scorers among the entire pilot group on all three types of mental alertness tests during preexposure and exposure periods.

In addition to the above, there were two other deaths among subjects during the study period. One mixing-plant worker committed suicide by drinking parathion. His test scores were near the lower range of normal. The details of this interesting case will be presented elsewhere.[10]

One control subject, chosen because of his lack of exposure to pesticides, died of a heart attack. There is no evidence that spray chemicals were involved in his death.

Clinical Studies.—Cases of organic phosphorus poisoning were studied in which combinations of various degrees of mental effects and physical signs and symptoms were noted. No cases with mental effects in the complete absence of physical signs or symptoms were noted. The following case reports are illustrative of definite mental effects in patients with minimal physical signs.

Report of Cases

Case 1.—A 17-year-old boy worked as a loader for an aircraft insecticide application service for about six weeks in the summer of 1960. His job involved 16 to 18 hours of duty seven days a week, loading airplanes with the following organic phosphorus insecticides: parathion, tetraethylpyrophosphate, demeton, and, especially in the last work week, Phosdrin. This work was much harder than that to which he was accustomed.

On Aug 2, 1960, the subject reported to our laboratory for a routine blood cholinesterase determination. He had been urged to do this as a precaution by his father, an entomologist. This boy's blood cholinesterase level was quite low—erythrocytes, 0.10 and plasma, 0.27Δ pH/hr. Although this young man did not consider himself ill, questioning elicited a history of minimal signs and symptoms which could have been due to his physical exhaustion and loss of sleep or to mild poisoning by organic phosphorus insecticides. He stated that by the third week of work he had noted twitching of his eyelids but, like subsequent mild symptoms, he did not consider this sufficiently annoying to warrant special attention. There was slight nausea, anorexia, and weight loss of 5 lb (2.3 kg) during this period. Sometime after the third week, frequent dizziness began, especially when he stood erect suddenly. On Aug 1, 1960, he almost drove his car into a telephone pole. He had noticed slowing of driving reactions and complained of a loss of sense of timing. He was unable to participate readily in his companions' activities.

He had excessive sweating restricted to forearms and legs, suggesting topical effects from absorption of organic phosphorus insecticide in areas of greatest exposure. The only unusual dermal contamination was spillage of parathion concentrate in one shoe about a week earlier. This was promptly washed off, and the shoes discarded. There had been slight paresthesia noted only on the hands. During the days preceding his examination he had encountered some difficulty in focusing his eyes. His eyelids felt very weak; it was difficult to open his eyes or keep them open; he had some minor visual disturbances, including one brief episode of diplopia. He denied excessive salivation or miosis. There were no respiratory signs or symptoms as often occur in loaders and other persons over-exposed to the vapors of Phosdrin. There was a slight cough, but no pain or tightness in the chest nor dyspnea. No intense headache could be recalled but two days earlier there had been a slight, brief headache which disappeared without treatment. This man had dreamed more than usual during this period but denied a change of dreaming pattern to nightmares or hallucinations. There was no apparent change in temperament, emotional affect, memory, or judgment.

On his initial visit the young man was given the Gersoni U test and the EX test, on both of which his scores were quite poor. At parental insistence, he quit his job, rested, and had no further exposure to insecticides. Three days later, his scores had improved markedly; this level of performance was maintained on subsequent tests (Table 4). Although no preexposure scores are available, it is obvious that this man's performance on his first test was below the expected level of attainment for his age and intelligence. His improved subsequent performances confirmed this impression.

Although the blood cholinesterase activity was depressed severely, the only sign or symptom which could not be attributed to sleep deprivation or physical exhaustion alone was excessive local sweating and paresthesia, effects known to result from local dermal exposure to certain organic phosphorus insecticides. Even so, the fact that the subject was rested from ten hours of sleep seems to rule out sleep deprivation as an explanation for his poor initial performance on the mental alertness test.

Case 12.—This 27-year-old sprayman for a pest control company suddenly became confused after six weeks of spraying trees with parathion using a hand gun. He broke the nozzle from his spray hose. While attempting to explain this to his boss, he developed a sense of dual personality, transposing himself outside his own body, watching and listening to himself as he talked. He felt that the employer appeared incredibly younger and the outline of his body was indistinct and fuzzy. His co-worker noted that he became more confused and ataxic. He was completely amnesic from the time of onset until recovery in the hospital about one hour later. Upon examination at the hospital he appeared to be entirely normal except for confusion and weakness. After a small dose of atropine (0.2 mg), the patient quickly began to improve, and shortly afterwards he was given pralidoxime chloride (0.5 gm) intramuscularly. The confusion continued to clear and he was essentially normal a few hours later. Blood cholinesterase activity was never depressed below normal range. Maximal *p*-nitrophenol excretion was 0.45 ppm at 5.5 hours after onset. Excretion of this parathion metabolite decreased to 0.13 and 0.07 ppm on the second and third days, respectively. Although mental confusion was the first recognized and predominant sign of intoxication, the patient had had a headache for at least two days, and nausea without vomiting for more than one day, and paresthesia just before onset. During a daytime nap 30 hours after onset, he experienced a bad dream about his poisoning.

The test scores of 11 individuals poisoned by organic phosphorus compounds, including case 1, are given in Tables 4 and 5. In the nine instances in which comparisons were available, the subject scored poorer in the acute or early recovery stage of poisoning and then showed progressive improvement in achievement during and after convalescence.

Comment

Of the numerous drugs that have been tested for effects on mental alertness, caffeine and the amphetamines have apparently been studied most thoroughly.[14] In a review, Weiss and Laties concluded that caffeine had little or no effect on reaction time or coordination, but impaired hand steadiness; the amphetamines lowered reaction time and improved coordination and hand steadiness, especially in fatigued subjects. Both caffeine and the amphetamines counteracted, to some extent, the decrement of motor performance produced by alcohol.

All three of the tests used in the present study were selected because of proven sensitivity in measuring mental alertness in persons deprived of sleep.[17] Some workers favor subject-paced tests (such as the Gersoni U test and the EX test) feeling that results of lapses of attention are cumulative in subject-paced tests, no matter when the lapses occur during the test whereas, in an experimenter-paced test, the lapses must correspond more or less exactly with the randomly distributed stimuli to be recorded.

Other groups prefer experimenter-paced tests, feeling that it is necessary to present discrete stimuli to the subject in "lapse of attention" periods to study the distribution, duration, and other characteristics of these periods.

It is generally agreed that any test of mental alertness to detect lapses of attention should be simple and that all stimuli, whether appropriate or not, should produce minimal arousal. For example, color should be pastel, light should be dim, and sound should be soft. By the same token, any test involving learning should be avoided because the act of learning provokes attention.

People with lapses of attention generally do not show much decrease in accuracy of performance.[18] Sleep-deprived subjects show slowing of performance in self-paced tests as well as an increase in the number of abnormally long reaction times and failures to respond to appropriate stimuli in experimenter-paced tests. However, even under the worst conditions, they continue to make many rapid and otherwise appropriate reactions.[18]

Confusion is rather common in those manifestly ill of organic phosphorus poisoning. The distinction between presence or absence of physical illness may not be important because confusion may make a victim fail to seek medical help for a condition which would otherwise alarm him. He may be unable to give a good history, even to the extent of not knowing his own name or the nature of his exposure. He may fail to take medical advice either because he is argumentative or because he is passively euphoric. Whether other signs and symptoms are mild or are completely absent, mental confusion would obviously be a great danger to anyone who happened to develop it while operating machinery, especially aircraft.

The difficulty may take the form of loss of judgment or of automatism, the former evidenced by complete indifference to recognize danger; the latter resembling sleepwalking. One pilot who lived through a crash reported that he had seen the obstacle which he struck but just did not care. Some pilots, usually cautious, have attempted maneuvers that their colleagues say would not be attempted by a rational person. Illustrating a sort of automatism, one applicator was seen pouring coffee into and over a sugar bowl instead of into his cup. In his case, fatigue was present but difficult to evaluate. Some men with confusion had a little warning through the appearance of mild symptoms of the usual kind. Others recalled no warning; their associates detected no illness until after confusion was evident. The suspected cases have been associated with several compounds; therefore, it would be unwise to assume that any organic phos-

TABLE 4.—*Performance on Gersoni U Test and EX Test for Individuals Poisoned by Organic Phosphorus Insecticides*

		Gersoni U Test			EX Test		
Case No.	Clinical Status	Errors of Omission	Errors of Commission	Lines Completed	Correct Answers	Incorrect Answers	No. of Questions Answered
1	Mild poisoning	30	6	68.2	62	55	117
	Recovery I	7	1	64.5	105	16	121
	Recovery II	10	0	68.6	111	4	115
2	Mild poisoning	13	0	77.3	—	—	—
3	Mild poisoning	1	1	50.9	127	7	134

TABLE 5.—*Performance on Complex Reaction Time Test for Individuals During Acute Stage of Poisoning by Organic Phosphorus Insecticides and After Recovery*

		Performance on Complex Reaction Time Test							
Case No.	Clinical Status	Errors of Omission	Errors of Commission	Responses Earlier Than 0.35 Sec	Responses Earlier Than 0.40 Sec	Responses Earlier Than 0.50 Sec	Responses Later Than 0.50 Sec	Responses Later Than 0.60 Sec	Mean Reaction Time (Sec)
4	Mild poisoning	0	2	1	—	23	30	—	0.58
	Recovered	0	1	1	—	53	9	—	0.49
5	Mild poisoning	0	0	13	—	40	10	—	0.49
	Recovered	1	3	12	—	54	9	—	0.47
	Recovered	0	3	11	—	61	9	—	0.48
	Recovered	0	2	12	—	57	9	—	0.48
6	Severe poisoning	2	2	2	—	28	12	—	0.54
	Partially recovered	0	3	2	—	27	22	—	0.58
	Recovered	2	1	1	—	25	16	—	0.51
	Recovered	1	0	8	—	55	8	—	0.48
	Recovered	4	0	5	—	61	5	—	0.47
7	80% ChE depletion	0	14	—	5	—	—	15	0.53
	Light exposure	0	11	—	17	—	—	10	0.48
	Normal	0	5	—	20	—	—	8	0.46
	Normal	1	8	—	22	—	—	8	0.46
8	80% ChE depletion	1	12	—	7	—	—	4	0.49
	Light exposure	0	10	—	19	—	—	2	0.45
	Normal	0	6	—	17	—	—	21	0.50
	Normal	0	8	—	26	—	—	2	0.44
9	80% ChE depletion	0	0	—	3	—	—	1	0.50
	Normal	0	0	—	15	—	—	12	0.46
	Normal	0	0	—	15	—	—	8	0.38
10	Mild poisoning	6	6	—	5	—	—	28	0.56
	Normal	0	4	—	38	—	—	11	0.44
	Normal	1	0	—	14	—	—	23	0.52
11	Mild poisoning	1	1	—	0	—	—	79	0.77
	Normal	2	9	—	14	—	—	73	0.70
	Normal	0	2	—	17	—	—	54	0.59
48 unexp persons	Control group Mean value	0.33	4.7	8.8	—	42.0	16.7	—	0.51
7 unexp persons	Control group Mean value	0.90	11.9	—	19.7	—	—	19.7	0.50

phorus compound cannot produce the same effect.

If the mental effects resulting from organic phosphorus exposure occur suddenly, test results may become abnormal suddenly, so it would be necessary to make the test at a specific time to obtain positive results.

Of the six parameters measured on the EX and Gersoni U tests in this study, only errors of omission and total lines completed on the Gersoni U test showed any indication of decreased performance correlated with exposure to organic phosphorus insecticides. However, the similarity of errors of omission for exposed and control groups seems to negate the effect suggested by the good correlation between performance judged by this standard and exposure for the various occupational groups. The only difference between exposed and control groups during the exposure period not explained by a similar difference during the nonexposure period was found in the lines completed on the Gersoni U test. Even then, there was no gradation of score between the various occupational groups correlated with the degree of their exposure. On the basis of these results, it is concluded that no measurable difference in performance on the EX or Gersoni U test correlated with exposure to organic phosphorus pesticides was demonstrated in the survey group.

The results of the complex reaction time test did not indicate that exposure to organic phosphorus insecticides had any significant effect on mental alertness.

However, these tests did indicate a decrease in alertness in the aircraft loader (case 1) discussed above. It is impossible to be certain that this young man's impaired test performance resulted from exposure to organic phosphorus pesticides, but this possibility must be considered strongly. Test results from 11 cases of organic phosphorus poisoning corroborated the opinion that these compounds do affect mental alertness when exposure is sufficiently great.

These test results are generally consistent with the accepted idea that organic phosphorus compounds may affect mental alertness if absorbed in amounts great enough to cause clinical signs or symptoms of systemic illness. Case No. 1 illustrated minimal physical illness associated with easily measurable mental effects. No cases were seen in which only mental effects occurred. The lack of occurrence of this phenomenon in our series of cases seems to indicate that mental effects occur rarely, if ever, in the complete absence of physical signs and symptoms.

Data on aircraft accidents sustained by cropduster pilots have been compiled by the Civil Aeronautics Board.[3] There were 314 aircraft accidents involving 57 fatalities reported for aerial applicators during 1959, the latest year for which statistics are available. The total accident rate in aerial application work (35.69 accidents per 100,000 flight hours) was essentially the same as for all general aviation flying * (36.80 accidents per 100,000 flight hours). However, the fatal accident rate was higher for application work (6.47 fatal accidents per 100,000 flight hours) than for all general aviation flying (3.63 fatal accidents per 100,000 flight hours). The higher incidence of fatalities in cropduster accidents may be due partly to the fact that in application work pilots customarily fly near the ground carrying heavy loads, and so are less able to take corrective action when mechanical failure or other difficulty occurs. There has been little or no change in the rate of cropduster accidents during the period 1951 to 1959. The apparent slight decrease in the rate of all accidents is difficult to interpret in view of the fact that the rate of fatal accidents remained essentially constant.

The Figure shows the rate of total and of fatal aircraft applicator accidents for 1951 through 1959 along with production of organic phosphorus pesticides in the United States for the same period.[12] The constancy of the accident rates during this time when the production and use of the organic phosphorus pesticides was increasing rapidly suggests that exposure to these chemicals was not a major cause of aircraft accidents involving cropduster pilots. However, such statistical comparisons cannot exclude the possibility that these insecticides contribute to a limited number of accidents.

The relationship, implied by Gershon and Shaw,[6] between pesticides and the etiology of a different kind of mental effect has been discredited on an epidemiologic basis.[1,2,6] Sixteen persons (14 men and two women) exposed for between 1½ and 10 years to organic phosphorus insecticides in Australia were alleged by these authors to have schizophrenic and depressive reactions with severe impairment of memory and difficulty in concentration. In one group of greenhouse technicians, the number allegedly affected was eight of ten exposed workers. Although organic phosphorus insecticides have been used almost all over the world, essentially all cases exhibiting this symptom complex occurred in a small area and were reported by one group of authors. An additional point involves magnitude of exposure. It is not apparent that the patients reported by Gershon and Shaw had exposures of unusual magnitude. Some of these workers, including three scientific officers (cases No. 9, 10, and 15) who were studying the efficacy of the sprays, apparently had little exposure during actual use but were exposed to residues. Hayes[8] pointed out that when large samples are considered, poisoning can be expected to appear most quickly, most frequently, most diversely, and most severely in persons most extensively exposed.

After publication of the report from Australia by Gershon and Shaw, a survey was undertaken in one hospital in an area of

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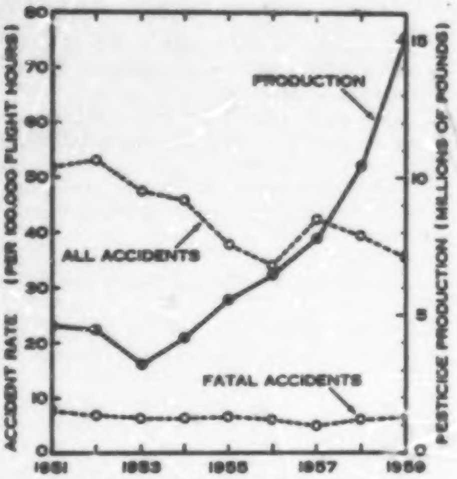
* General aviation flying includes all flying except that by military aircraft and by commercial planes engaged in transportation of passengers and freight.



Total and fatal aircraft accident rates for applicators in relation to production of organic phosphorus pesticides in the United States.

New Zealand where organic phosphorus compounds were used extensively. It was concluded that these insecticides had no significant role in the etiology of illness for any patients admitted to the hospital during the survey.[10]

The experimental studies of Rowntree et al [11] indicate that when exposure of sufficient magnitude is involved the organic phosphorus compounds can aggravate psychiatric illness and induce mental effects in normal persons. These investigators administered diisopropyl fluorophosphonate to 26 psychotics (17 schizophrenics and nine manic-depressives) at total dosages of 13 to 43 mg over periods of 7 to 37 days. In six schizophrenics, the psychosis was activated, involving reappearance of florid symptoms which had characterized the onset of illness. There was no evidence of impairment of consciousness. The changes persisted for some months after withdrawing the diisopropyl fluorophosphonate. In the manic-depressive group, in general, mania was lessened and depression increased by dosage with diisopropyl fluorophosphonate; insomnia and increased dreaming were induced. Ten normal controls developed characteristic altered emotional affect with depression, irritability, lassitude, apathy, dejection, and unhappiness. Electroencephalographic changes were described including lowering of amplitude associated with diminution of α-activity.

However, Fiamberti [4] described improvement in schizophrenics produced by intravenous injections of acetylcholine.

Summary

Tests of mental alertness were conducted during 1960, 1961, and 1962 on groups of people with varying degrees of exposure to organic phosphorus pesticides and on controls.

In 1960, two different pencil and paper tests—the Gersoni U test and the EX test—were used. On both tests unexposed individuals varied widely in levels of attainment. However, for a given individual the scores attained on the same test at different times were fairly uniform. Only a minor amount of learning occurred. There was little or no difference among the various exposure groups on five of the six parameters measured. However, with respect to lines completed on the Gersoni U test, the exposure group as a whole showed a poorer score during the exposure period compared with the nonexposure period while the control group made a better score during the "exposure" period than during the "nonexposure" period. This one difference was the only indication from the 1960 tests that exposure to organic phosphorus insecticides modified mental alertness in clinically well workmen.

In 1961 and 1962, a complex reaction time test was used. In this test, also, performance varied widely among different people, but performance for a given individual was relatively constant at different times. Little learning was evident. Both exposed and control groups scored about as well or better during nonexposure than during exposure periods. The differences were small for both exposed and control groups and were as large, or larger, for the latter as for the former. There was no indication from results of the complex reaction time test that exposure to organic phosphorus insecticides at levels insufficient to produce clinical illness had any important effect on mental alertness.

Three of the pilots in the survey crashed during the period of participation. There was no evidence that pesticide exposure was involved as the cause of these incidents.

In the clinical study of 187 cases of suspected organic phosphorus poisoning, each case was evaluated on the basis of the relative severity of somatic illness and of mental effect. There were no cases in which mental effect was noted in the complete absence of physical signs or symptoms of illness. Two cases are reported in which an effect on mental alertness was the presenting and/or predominating symptom.

The mental alertness tests were given to 11 individuals who had shown symptoms of poisoning by organic phosphorus insecticides recently. In the nine instances in which comparisons were available, the poisoned subjects scored poorer in the acute or early recovery stage of poisoning and then showed progressive improvement during and after convalescence.

These test results are consistent with the accepted opinion that organic phosphorus compounds may affect mental alertness if absorbed in amounts great enough to cause clinical signs or symptoms of systemic illness. No cases were seen in which mental effects occurred alone.

The constancy of accident rates for cropduster pilots during a time when production and use of organic phosphorus pesticides was increasing rapidly suggests that exposure to these chemicals was not the major cause of aircraft accidents involving cropduster pilots.

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Teratogenic Effects of Cadmium in Rats

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ABSTRACT The teratogenic effects of subcutaneous injections of cadmium in CD rats were investigated. Injections of 4–12 mg/kg $CdCl_2$ on 4 consecutive days beginning on days 13–16 of gestation resulted in a dose-related rise in the fetal death rate, decrease in fetal weight, and increase in the rate of anomalies. The anomalies included micrognathia, cleft palate, clubfoot, and small lungs. The lung/body weight ratios were significantly reduced in fetuses of animals injected with 8 mg/kg $CdCl_2$ on days 14–17 of gestation. The data indicate that this was a specific retardation and not merely a reflection of differential organ growth rates and overall fetal growth retardation.

Cadmium is a ubiquitous air and water pollutant, a variety of whose toxic effects in mammals have been extensively studied (Flick et al., '71). The effects of cadmium on the placenta and fetus have been investigated recently. Destruction of the placenta and fetal death after single maternal subcutaneous injections were demonstrated in Wistar rats (Parizek, '64) and Swiss Albino mice (Chiquoine, '65), and the production of extensive malformations was noted in hamster fetuses after maternal intravenous administration (Ferm and Carpenter, '67). Several experiments indicated that the teratogenic and fetal toxic effects of cadmium may be prevented by the simultaneous injection of zinc (Ferm and Carpenter, '67, '68) or selenium (Parizek et al., '68; Holmberg and Ferm, '69).

The present studies were initiated to determine the sublethal effects of cadmium on rat fetuses. Morphological defects and specific organ growth changes resulting from cadmium administration are described.

MATERIALS AND METHODS

CD rats (randombred) were used. Females were considered 1 day pregnant at demonstration of sperm in the vaginal smear after overnight caging with males. The animals were kept in individual wire cages in a constant-temperature room (22 ± 2°) with controlled lighting (15 h of light) and fed commercial lab chow and water ad libitum. Pregnant animals were each injected sc with 4, 6, 8, or 12 mg/kg $CdCl_2$ in physiological saline daily on 4 consecutive days beginning on gestation day 13, 14, 15, or 16. Controls received saline. All injections were 0.1 ml in volume, and were made between 10 and 11 AM.

Animals were killed by decapitation on day 21 of gestation, the uteri removed and opened, and resorption sites, dead fetuses, and live fetuses noted and recorded. Live fetuses were weighed and preserved in Bouin's solution. After 3–5 days they were examined for external defects and then necropsied. In experiments in which fetal organs were weighed, this was done immediately after the fetuses were removed from the uterus.

Calculations were based on the litter as the unit and standard deviations therefore represent the variation between litter means. Fetal organ weight data are expressed as percentage of total fetal weight.

Fetal weight and organ/body-weight ratios were analyzed using analysis of variance procedures. The method of unweighted means was employed, with treatments and replicates as two factors of interest. Fetal anomalies and mortality were analyzed using the Mann-Whitney U test.

RESULTS

Fetal mortality

A dose-related fetal mortality response

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was established for $CdCl_2$ administered on days 14–17 of gestation. The fetal mortality increased significantly ($P < .01$) and was related to dose, ranging from 4.4% at 4 mg/kg to 51.6% at 12 mg/kg (table 1). Administration of 8 mg/kg on various days of gestation revealed that the fetuses were most sensitive when treatment was begun on day 14 or 15 (table 2). Fetal mortality following treatment begun on day 13 was significantly lower than after other treatment periods ($P < .01$) although higher than control values ($P < .05$). There was also an indication that fetal mortality was reduced when treatment was begun on day 16 ($P < .10$). A significant number of litters of animals treated with 8 or 12 mg/kg were totally resorbed.

Fetal weight

The administration of 6–12 mg/kg $CdCl_2$ significantly ($P < .01$) reduced fetal weights. Fetal weight dropped from the mean of 4.4 g in controls to 2.8 g in the group treated with 12 mg/kg on days 14–17 and 2.6 g in the group injected with 8 mg/kg on days 15–18 (tables 1, 2).

Malformation

The frequency of litters containing one or more abnormal fetuses and that of abnormal fetuses were significantly ($P < .01$) increased after treatment with 6 mg/kg $CdCl_2$ or greater (tables 1, 2). The phenomenon was dose-related after treatment on days 14–17. The most common anomalies seen were lower-jaw defects, cleft palate, and hypoplastic lungs.

Lower-jaw defects occurred in various degrees, from agnathia to slight mandibular shortening. The frequency of affected fetuses after treatment on days 14–17 ranged from 3% with 4 mg/kg to 76%

TABLE 1

Effects of various doses of $CdCl_2$ injected sc on days 14–17 of gestation

Dose	Total no. of litters	No. of litters with live fetuses	Avg. live	Fetal dead and/or resorbed	Fetal weight (g)	Abnormal litters	Abnormal fetuses	Anomalies	
								Jaw defects	Cleft palate
mg/kg				% [2]	*mean ± SD*	% [3]	% [2]	% [2]	% [2]
0 [1]	17	17	8.4	5.2	4.5 ± 0.4	5.9	0.8	0.0	0.0
4	15	15	9.6	4.4	4.1 ± 0.6	6.7	4.4	4.4	1.1
6	14	13	7.1	29.3	3.3 ± 0.6	61.5	34.8	30.0	8.5
8	30	20	5.5	47.9	3.3 ± 0.8	85.0	47.9	47.4	11.4
12	13	9	5.4	51.6	2.8 ± 0.6	100.0	69.7	69.7	26.0

[1] Saline-injected controls.
[2] Mean of the litter percentages.
[3] An abnormal litter is defined as a litter with one or more abnormal live fetuses.

TABLE 2

Effects of sc injections of 8 mg/kg $CdCl_2$ during different periods of gestation

Treatment days	Total no. of litters	No. of litters with live fetuses	Avg. live	Fetal dead and/or resorbed	Fetal weight (g)	Abnormal litters	Abnormal fetuses	Anomalies	
								Jaw defects	Cleft palate
				% [2]	*mean ± SD*	% [3]	% [2]	% [2]	% [2]
Saline									
13–19 [1]	36	36	9.0	2.8	4.4 ± 0.4	2.8	0.4	0.0	0.0
$CdCl_2$									
13–16	29	27	8.3	11.5	3.2 ± 0.7	70.4	27.5	26.5	6.9
14–17	30	20	5.5	47.9	3.3 ± 0.8	85.0	47.9	47.4	11.4
15–18	10	5	2.5	72.2	2.6 ± 0.7	100.0	55.0	55.0	0.0
16–19	10	8	7.9	34.8	2.9 ± 0.6	75.0	30.1	30.1	0.0

[1] Since no significant differences were found between control groups receiving saline injections during differing periods of gestation, these groups are lumped together.
[2] Mean of the litter percentages.
[3] An abnormal litter is defined as a litter with one or more abnormal live fetuses.

TABLE 3

Body weight of fetal organs from litters treated with 8 mg/kg $CdCl_2$ during different periods of gestation

Compound	Days of administration	No. of litters	Day killed	Fetal weight (g)	Organ/body-weight ratio (%)		
					Lung	Heart	Kidney
						mean ± SD	
Saline	13–17 [1]	12	20	2.7 ± 0.3	3.7 ± 0.4	0.61 ± 0.04	0.81 ± 0.03
Saline	13–17 [1]	10	21	4.3 ± 0.3	3.4 ± 0.2	0.62 ± 0.04	0.88 ± 0.07
$CdCl_2$	13–16	13	21	3.4 ± 0.5	2.5 ± 0.6	0.65 ± 0.04	0.82 ± 0.08
$CdCl_2$	14–17	10	21	3.5 ± 0.7	2.5 ± 0.8	0.65 ± 0.06	0.83 ± 0.12

[1] Since no significant differences were found between control groups receiving saline injections during differing periods of gestation, these groups are lumped together.

with 12 mg/kg. Approximately 50% of fetuses exposed to 8 mg/kg beginning on day 14 or 15 had lower-jaw defects. A smaller percentage (approximately 30%) occurred when treatment was begun on day 13 or 16. Cleft palate, which was always associated with jaw defects, occurred in 1% of the fetuses after 4 mg/kg on days 14–17. The percentage rose with increasing dose, until it was present in 26% of the fetuses treated with 12 mg/kg. Cleft palate was not induced when treatment was begun later than day 14.

Clubfoot, open eyelids, or both were present in some of the groups. Litters of animals receiving 8 or 12 mg/kg $CdCl_2$ beginning on day 13 or 14 had 4% hindlimb clubfoot. In approximately 40% of the cases the clubbing was bilateral. Open eyelids were present in 4% of the fetuses exposed to 8 or 12 mg/kg on days 14–17.

Hypoplastic lungs

In order to assess the relative sizes of fetal organs, a series of experiments were performed in which fetal kidneys, hearts, and lungs were weighed (table 3). Control litters at 20 and 21 days of gestation were compared to 21-day $CdCl_2$-treated litters. There were no significant differences in the organ/body-weight ratio of the controls of different ages; and there were also no significant differences in heart and kidney/body-weight ratios in treated and control 21-day fetuses. A significant ($P < .01$) reduction in lung/body-weight ratios occurred in the cadmium-treated groups. Lungs of control animals represented 3.4% of the total body weight whereas those of fetuses exposed to 8 mg/kg beginning on day 13 or 14 were 2.5% of the body weight.

DISCUSSION

The experiments reported indicate that sc injections of $CdCl_2$ in CD rats had various fetotoxic effects. Increased fetal mortality, decreased mean fetal weight, and a number of anomalies including cleft palate, open eyelids, and lower-jaw defects were produced. It is not known whether these toxic manifestations are the result of direct action on the fetus, maternal or placental effects, or a combination of these factors. Although the placenta is an effective barrier against the transfer of small amounts of maternally administered cadmium (Berlin and Ullberg, '63), larger amounts will enter the fetus (Ferm et al., '69).

Fetal growth retardation or an increased number of "runts" has been shown to occur after maternal administration of heavy metals such as tellurium (Garro and Pentschew, '64), lead (Schroeder and Mitchener, '71), and arsenic (Hood and Bishop, '72). The inhibition of lung growth in the present study, however, was not the result of a difference in overall fetal and lung growth rates associated with general growth retardation since the lung/body-weight ratios remained the same in control 20- and 21-day fetuses despite a large difference in mean fetal weight between these two groups (table 3). Experimentally induced lung hypoplasia did not result from maternal heavy-metal treatment, although it did from vitamin A (Warkany et al., '48; Warkany and Roth, '48) and vitamin E (Verma and King, '67) deficiencies. The relation(s) of cadmium to the fetal lung are unknown although cadmium inhibits oxidative metabolism in the alveolar macrophages of adult rat lungs (Mustafa et al., '71).

The experiments reported here showed that cadmium injected into CD rats is a potent teratogen and causes growth retardation of the fetal lung.

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TOXICOLOGY PROGRAM

Effects of Pesticides on Man

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Useful information on the effects of pesticides on man may be obtained from use experience data, accidental poisoning cases, and human volunteer studies. Indirect data which can be extrapolated to apply to man with varying success can be obtained from studies with laboratory animals.

In the usual course of manufacturing, formulating, packaging, and applying a pesticide, a number of workmen will receive significant exposure. It has been pointed out that, on the average, poisoning can be expected to appear most quickly, most frequently, most diversely, and most severely in those persons most extensively exposed (AMA Committee on Pesticides, 1956). For this reason, the study of men with prolonged occupational exposure to pesticides is of particular interest. However, the general population, exposed to small quantities of these chemicals as a residue in the diet or elsewhere in the environment, represents by far the largest group for study. Study of the general population is complicated by the lack of, an unexposed or less-exposed control group for use as a comparison.

Derivation of meaningful information on the health effects of pesticides from accidental poisoning cases and use experience data is dependent upon determining the exposure level involved and establishing that a toxic effect did or did not occur.

One of the major problems in studies of this sort is to determine the exposure and absorption levels. Clearly, demonstration of lack of harm to a certain individual or group is essentially meaningless unless some measure of the degree of exposure or absorption is available. For use experience data and poisoning cases, determination of dosage level may be difficult. The dosage level is, of course, accurately known in human volunteer studies involving controlled exposures.

In some instances it is possible to derive a meaningful figure for the maximum tolerated dosage or for the dosage which produced poisoning.

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In other circumstances, although use experience with the chemical may be available or poisoning attributed to it may have occurred, no actual figure is available for the dosage-effect relationship. In certain other instances, although no dosage value can be obtained directly, it is possible by indirect means to obtain a figure. For example, in the work of Ortelee (1958) with workers in DDT-formulating plants, although the amount of DDT exposure was not directly known, an estimated figure could be derived from the DDA excretion levels of the workers in comparison with DDA excretions of human volunteers exposed to known daily doses of DDT.

There is also a qualitative difference between data obtained from use experience and data derived from poisoning cases or human volunteer studies. In the latter instances, exposure is usually to a known single, specific compound. However, use experience is generally complicated by the fact that exposure is to more than one compound.

Exposure to the chlorinated hydrocarbon pesticides can be estimated from the storage level of the compounds or of their metabolites in adipose tissue (Mattson *et al.*, 1953) or, in certain instances, from excretion of biotransformation products in urine (Cueto and Hayes, 1962). The development of gas chromatography as an extremely sensitive analytical technique has made possible studies of pesticides or their metabolites in adipose tissues at low levels. Further applications of the methods of gas chromatography to samples from environmentally or occupationally exposed individuals can be expected to produce additional significant data on pesticide exposure levels.

The chlorinated hydrocarbon compound which has been studied most carefully in regard to storage in man is DDT. In the general population of the United States, the average storage of DDT is about 5 ppm, and the concentration of all DDT-derived material, expressed as DDT, is about 12 ppm (Hayes *et al.*, 1958; Quinby *et al.*, 1965). Abstainers from meat (Hayes *et al.*, 1958) and Eskimos (Durham *et al.*, 1961) store less than the general population, whereas agricultural applicators store about three times as much as the general population and formulators may store more than 600 ppm of DDT and more than 1000 ppm of DDT-derived material. Studies both in experimental animals (Ortega *et al.*, 1956) and in man (Hayes *et al.*, 1956) show that the storage level of DDT in fat does not increase indefinitely during continuous exposure but, instead, reaches a plateau characteristic of the particular level of intake. Surveys carried out in 1954–1956 (Hayes *et al.*, 1958), in 1961–1962 (Quinby *et al.*, 1965), and in 1963 (Hoffman *et al.*, 1964) showed that no increase had occurred in DDT storage among people in the United States since 1950, when Laug and his associates (1951) measured it for the first time.

More recent studies have shown that dieldrin (Hunter *et al.*, 1963; Dale and Quinby, 1963; Hoffman *et al.*, 1964) and lindane (Dale and Quinby, 1963; Hoffman *et al.*, 1964) also are stored in people without occupational exposure. It would be helpful to have some of the types of information which are cited above for DDT available for these other compounds. For example, one would like to know whether or not the storage of these other compounds also is at equilibrium, as DDT appears to be. Further, the possibility of storage of other pesticides in fat in the general population should be investigated with the most sensitive methods available.

For DDT, the daily intake associated with the fat storage level characteristic of the general population has been determined. Thus, Walker and his associates (1954) made studies on complete meals and estimated the average DDT intake at 0.184 mg per person per day. Comparable data for other stored pesticides would be useful. At the present time only qualitative data on dietary content are available for compounds other than DDT (Mills, 1963).

Exposure to the organic phosphorus compounds generally can be determined by measurement of blood cholinesterase activity (Sumerford *et al.*, 1953). Exposure to parathion and other organic phosphorus compounds which on hydrolysis form *p*-nitrophenol or one of its congeners can be estimated by determination of these phenolic compounds in urine (Arterberry *et al.*, 1961). Although applicable to a restricted group of compounds, this test has proved to be a more sensitive measure of absorption than is cholinesterase inhibition. A urine test based on excretion of organically bound phosphorus would be desirable since metabolites of this type are, of course, common to all the compounds in this chemical group. However, practical use of a test for organic phosphorus has been confined to malathion (Mattson and Sedlak, 1960; Hayes *et al.*, 1960).

Exposure to the carbamate insecticides can also be estimated by use of blood cholinesterase level (Witter, 1963). It is important to note, however, that the carbamates form reversible combinations with cholinesterase. It is necessary that special precautions be taken in the measurement of cholinesterase activity of persons or animals exposed to these compounds; otherwise, erroneous results will be obtained. The systematic inaccuracy is always in the direction of causing the sample to appear to be more normal than is actually the case.

The measurement of exposure of workers to pesticides has received considerable study (Durham and Wolfe, 1962). In addition to indirect measurements of exposure, such as storage or excretion of the pesticide or its metabolite(s) or pharmacologic effect, which have been discussed above, direct methods also have been used. The direct methods involve the use of some mechanism to entrap the toxic material as it comes in contact with the workman during his exposure period. The amount of trapped toxicant as determined by chemical analysis, bioassay, or other method is then a direct measure of the particular exposure being measured.

Direct measurement of exposure of workers has been carried out in our laboratory by attaching absorbent patches on the skin of spraymen during actual spray operations (Durham and Wolfe, 1962). Respiratory exposure has been determined by analyzing the filter pads from each worker's respirator and by determining through air sampling the amount of toxicant in the air near the breathing zone of the operator. Measurements of this sort have been carried out for a number of pesticides and for workers doing various agricultural and public health jobs associated with spraying or dusting operations. It is interesting to note that there is very good correlation between respiratory exposure values determined from respirator pads and from air-sampling studies. The work to date indicates that, for most of these situations, exposure is greater by the dermal route than by the respiratory route. However, in evaluating the relative toxic hazard of contamination from these two routes of exposure, the difference in absorption must be taken into account. Thus, absorption of inspired material is more rapid and more complete than is absorption of the dermally encountered compound.

Exposure values derived in this way can also be used to obtain additional information (Durham and Wolfe, 1962). The total calculated exposure (for both dermal and respiratory routes), expressed as milligrams per kilogram, can be compared with dosage levels that produce different degrees of injury in experimental animals. However, precise animal data on respiratory toxicity are not usually available. Therefore, a weighting factor should be used to take into account the more rapid and more complete absorption of respiratory doses than of oral or, particularly, dermal doses of toxicant.

A refinement of this procedure has recently been suggested for determining exposure and, particularly, for studying the relative dermal and respiratory absorption of pesticides by exposed individuals (Durham and Wolfe, 1963). This refined procedure involves estimation of total absorption of the chemical from measurement of the compound or its biotransformation product(s) in the urine or other excreta. Respiratory absorption is assumed to be equivalent to respiratory exposure and is, therefore, total potential respiratory absorption. It is estimated on the basis of the concentration in air of the chemical under study and of an assumed tidal volume and respiratory rate for the exposed individual or by other applicable procedure. Dermal absorption is calculated by difference between total absorption and potential respiratory absorption. The value obtained through use of this method should represent a minimal value for dermal absorption.

The technique has been applied in our laboratory to exposure to parathion by orchard spraymen (Durham and Wolfe, 1963; Durham et al., manuscript in preparation). The results indicated that dermal absorption was greater than respiratory absorption under the conditions of the study. It would be desirable to have this procedure tested for other compounds and for parathion under conditions of use different from those of our study. Study of dust formulations of parathion for comparison with results on the liquid spray material would be of particular interest.

In connection with measurement and evaluation of degree of exposure, it is important to consider variation in response among individuals to these toxicants. A few approaches for experimental study of and possible explanation for individual variability have been proposed. For example, an increased susceptibility to primaquine and certain other drugs with hemolytic properties has been associated with a deficiency of the enzyme glucose-6-phosphate dehydrogenase (Beutler, 1959). A few cases suggest but do not prove that some people are hypersusceptible to organic phosphorus insecticides. The hypothesis is under investigation (Mountain and Stalinger, 1960) that congenital deficiency of blood plasma cholinesterase is correlated with possible hypersusceptibility.

Another possible problem of hypersensitivity to pesticides relates to the blood dyscrasias. There has been some fear expressed that pesticides were causing an increase in blood dyscrasias. However, study of statistics from the United States for the era of the modern synthetic pesticides indicates no increase in agranulocytosis, aplastic anemia, or purpura (Hayes, 1962). There has been some increase in the incidence of Hodgkin's disease and a striking increase in leukemia and aleukemia. However, there was no significant change in the rate of this increase between 1939 and 1959, and, most important, there has been no augmentation of this increase associated with the introduction of DDT or with the more recent introduction of the other synthetic pesticides. However, these statistics for the population at large do not rule out the possible occurrence of isolated individual cases in which blood dyscrasias may be induced by pesticides. In instances in which individuals develop

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blood dyscrasias in association with heavy exposure to pesticides, a dependable *in vitro* test for laboratory confirmation of the cause-effect relationship would be most helpful. The alternative procedures (a challenge dose of the pesticide suspected of causing the illness or a dermal patch test) are less desirable than an *in vitro* test.

Certain information on the toxicology of pesticides can be obtained only through studies on volunteers. To be sure, dosages for volunteers are generally restricted to lower levels than those actually encountered in use, and there are practical limitations on the duration of studies on volunteers. However, work with volunteers makes it possible to study the relation of absorption, storage, excretion, and physiological effect to an accurately known dosage. Some physiological functions, such as reaction time, visual acuity, and ability to judge distance, can be studied in animals only with so much difficulty that the results are hard to interpret even for the species under study.

A number of careful studies have been carried out in which human volunteers have been exposed experimentally to measured doses of pesticides. Various dosage routes, including oral, dermal, and respiratory, and single as well as repeated doses of toxicant, have been used in these studies. For a general discussion of human volunteer studies with pesticides and a detailed tabulation of published studies see the paper by Dr. W. J. Hayes, Jr. (this symposium, p. 329).

Volunteers were fed DDT dissolved in corn oil at doses of 0, 3.5, and 35 mg/man/day for periods up to two years (Hayes *et al.*, 1956, and manuscript in preparation). Since all the men were on an ordinary diet, these dosage levels represented about 1, 20, and 200 times the ordinary dietary level. No clinical effect associated with dosage was detected either by the men themselves or as a result of careful physical examination and laboratory testing. The storage of DDT in body fat and the excretion of DDA in urine were measured.

The oral toxicity to man of a number of organic phosphorus insecticides has been studied utilizing depression of blood cholinesterase level as the most sensitive measure of physiological effect. Compounds which have been studied in this manner have included Delnav (Weir and Keller, 1962; Frawley *et al.*, 1963), demeton (Upholt *et al.*, 1954), dimefox (Edson, 1956), dimethoate (Sanderson and Edson, 1964), EPN (Moeller and Rider, 1962), malathion (Mattson and Sedlak, 1960; Moeller and Rider, 1962), parathion (Edson, 1957; Rider *et al.*, 1958), and schradan (Edson, 1955). Studies have also been carried out on the possible potentiating effect on man of combinations of EPN and malathion (Moeller and Rider, 1962).

In other studies, excretion of *p*-nitrophenol and depletion of blood cholinesterase have been used to measure absorption and metabolism of parathion by volunteers (Funckes *et al.*, 1963; Hartwell *et al.*, 1964; G. R. Hayes *et al.*, 1964). These studies have shown that dermal absorption of parathion increased with increase in temperature (Funckes *et al.*, 1963). This finding correlates well with the observation of physicians and others in certain agricultural areas that poisoning from the organic phosphorus insecticides occurs more often in unusually hot weather than under cooler conditions.

These studies on volunteers (G. R. Hayes *et al.*, 1964) revealed a lack of toxic effect from large dermal doses of parathion although parathion applied to the skin of laboratory animals has shown high toxicity (for example, Gaines, 1960). In the studies on volunteers, the absorption of large amounts of parathion or its breakdown products following dermal dosing was confirmed by the finding of high levels of *p*-nitrophenol in the urine (up to 5.9 mg per 24-hour sample), but little or no associated cholinesterase depletion and no cholinergic signs or symptoms occurred. However, those workers were able under other conditions of dosage, particularly by the respiratory route or by a combined respiratory-dermal dosage, to produce significant cholinesterase depletion as well as serious poisoning in volunteers (Hartwell *et al.*, 1964; G. R. Hayes *et al.*, 1964). The apparent interspecies differences in rate or mechanism of absorption and/or in sensitivity to the chemical should be investigated further.

The critical threshold level for parathion absorption above which poisoning might be expected to occur should be exactly defined by measuring absorption rates (based on *p*-nitrophenol excretion) over intervals as short as possible. Thus, Arterberry and his co-workers (1961) were able to show a good correlation for both *p*-nitrophenol excretion and blood cholinesterase inhibition with variation in exposure level of asymptomatic workers as well as with severity of symptoms in poisoning cases.

The toxicity of aerosols containing malathion was investigated by Golz (1959). Groups of four men each received 84 exposures of 1 hour each, in 42 consecutive days at dosages of 0, 0.15, 0.6, and 2.4 g of malathion per thousand cubic feet of air. At no time did the men experience any cholinergic symptoms or signs, nor was there any significant or dose-related change in blood cholinesterase. The highest dosage, which was far greater than anything which would result from practical use, was moderately irritating to the nose and conjunctiva.

The safety of malathion dusting powder for louse control was tested by dusting the bodies and clothing of volunteers daily for 8 or 16 weeks with talcum powder containing up to 10% malathion (Hayes *et al.*, 1960). Complaints about odor and skin irritation were approximately proportional to the dosage. No change in blood cholinesterase was found except with the 10% powder. Urinary excretion of malathion-derived material was proportional to the dosage. No other changes attributable to malathion were noted, and the compound was considered safe for the proposed use. Subsequent field tests have confirmed the safety and effectiveness of malathion dusting powder for louse control (Barnes *et al.*, 1962; Shawarby *et al.*, 1963).

In connection with the proposed use of DDVP for malaria control and for the disinsection of aircraft, studies have been made of volunteers exposed to vapors of this volatile insecticide (Rasmussen *et al.*, 1963). At high vapor concentrations (0.40–0.66 μg/l) and a dosage schedule of 78 exposures of ½ hour each over a 14-day period, inhibition of plasma cholinesterase activity was significant. No other abnormalities were seen.

The effect on vision of TEPP applied locally to the eye was studied by Upholt *et al.* (1956). They found that instillation of 2 drops of a 0.1% solution in peanut oil caused maximal miosis, a decrease in light perception, and an increased depth of focus in the eye. When the application was unilateral, volunteers suffered a decrease in ability to judge distance, but this did not occur when the application was made to both eyes. The practical implication of this work was illustrated later by the experience of a pilot who had been applying methyl parathion and was having great difficulty landing his plane; he found that his vision cleared when he closed one eye, and he was able to land without further difficulty (Quinby *et al.*, 1958).

Harvey *et al.* (1951) studied physiologic effects and blood and urine levels of DNOC in volunteers given oral doses of this pesticide. This report established that symptoms of headache, lassitude, and malaise corresponded to blood levels of about 20 ppm of DNOC. The article

reveals In ~~summary,~~ it is apparent from what has been said above that a considerable amount of information on the effect of pesticides on man is available from use experience, from human poisoning cases, and from volunteer studies. It appears that more attention should be paid to obtaining toxicological information based on use experience associated with workmen occupationally exposed or residents living in proximity to pesticide-treated areas. Previous study has shown that many occupationally exposed workers suffer exposures of magnitudes which could not be safely applied to volunteers. However, studies must also be carried out on volunteers in order to obtain information based on accurately known dosages. Information is particularly lacking on the possible long-term effects of small, repeated doses of pesticides on man.

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